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HETEROSIS IN BARLEY

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I. INTRODUCTION.

THE cultivated varieties of barley have a low chromosome number ($2n=14$). They are autogamous and belong among the oldest of man's domestic plants. In thousands of generations the cultivated species have been closely dependent upon the activities of man, and it seems probable that the selection process in barley has been different from that in wild species. Such differences between wild species and old domestic ones may conceivably have influenced the phylogeny and, thus, the whole set-up of genes within the species.

In previous papers the present author has published a study of the F_1 of a large number of crosses between pure lines in some *Galeopsis* species (HAGBERG, 1952 a and b). These species are wild and mainly autogamous, with the chromosome numbers $2n=16$ and $2n=32$. It seemed to be of interest to make a similar study of the F_1's of a number of crosses between barley lines, for the sake of comparison, and the study here presented was started in 1945.

The problems to be studied in the investigation as a whole were listed in the papers mentioned and the general aim has directed also the planning of the present experiments. The following questions were especially the object of the studies: (1) Where is hybrid vigour to be found; in this case: is it to be found in barley and, if so, in which or what type of combinations? (2) Is the hybrid vigour equally marked in diploid and in tetraploid species? (3) Is there any relation between the degrees of geographical and ecological differentiation between the parent lines and the degree of hybrid vigour in F_1? (4) Is there any relation between the degree of reduction in fertility and that of hybrid vigour in F_1? (5) At which stages of development and in which characters does hybrid vigour become evident? (6) Is F_1 more variable than the parent lines? (7) Is there any monofactorial hybrid vigour?

In planning the work the intention was, besides the comparison between wild and domestic species, to use the barley material to elucidate some of the problems, which could not conveniently be studied in *Galeopsis*. In the first hand F_1 has been compared with the parent lines but in some crosses it has also been possible to compare

F_1 with F_2—F_6, or, from the point of view of inbreeding, I_0 with I_1—I_5. Finally, some crosses have been made between morphological X-ray mutants and their mother lines; the results of these latter crosses are published separately.

A number of crosses were made in 1945—1947 between different barley varieties in the assortment of the Swedish Seed Association. In 1946—1948 the F_1's of these crosses were studied and in these and the following years a restricted number of crosses have been used for a study of the inbreeding results. The latter part of the work has been carried out partly in collaboration with the Barley Division of the Seed Association; for the rest the material has been grown at the Svalöf branch station of the Genetics Institute of the University of Lund. Financial aid has been obtained from the Nilsson-Ehle foundation and from the Royal Physiographic Society of Lund.

II. MATERIALS AND METHODS.

Sixteen different varieties of spring barley have been used in the crosses. They all belong to different »species» within the section *Cerealia* of *Hordeum* (*cf.* ÅBERG, 1940). Most of them are two-rowed and *nutans*, but also some *erectum* and six-rowed varieties were included. One of the varieties, Gullkorn (Golden barley), K—A, has been used as standard and crossed with all the others. Gull is an old pure line variety, bred by HANS TEDIN (1913) by individual selection from a land variety from the Isle of Gotland. The variety has kept constant and uniform and seems to be well suited as a standard both in theoretical mutation experiments and in studies such as those here presented.

The varieties used are:

K—A	Gull, individual selection from a land variety,	2-rowed, *nutans*,	Gotland, Sweden
K—B	Bonus, cross-bred variety (Svalöf)	» »	»
K—C	Balder, » » » (Weibulls)	» »	»
K—D	Sv 0125, individual section from a land variety,	» »	Skåne »
K—E	Sv 45/362, » » » » » »	» »	Värmland »
K—F	Asplunds,	6-rowed, dense-eared	»
K—G	Svanhals, a very old variety from cross-bred German material, the selection made at Svalöf,	2-rowed, *erectum*	
K—H	Line from a German land variety	» *nutans*	
K—I	» » an English » »	» »	
K—J	» » a Russian » »	» »	
K—K	» » a Canadian » »	» »	
K—L	» » an Egyptian » »	» »	
K—M	» » a land variety from Smyrna	» »	
K—N	» » an Australian land variety	» »	
K—O	» » an Ecuadorian » »	6-rowed, lax-eared	
K—P	» » an » » »	» »	

The material derives from different parts of the world. Three (K—B, K—C, K—G) of the varieties are derived from cross-breeding, the other ones are more or less in the nature of lines from land varieties.

Crosses have been made within as well as between lines or varieties. The crosses were made in 1945—1947. In the first year the result was rather poor, mainly due to unsufficient technical skill (as to methods for crossing, *etc.*, *cf.* SMITH, 1951). In the last year the result was fairly sufficient, 4200 seeds being obtained from about 9000 emasculated and pollinated flowers. In all 43625 flowers have been emasculated and pollinated, giving 9932 seeds, about 200 per studied biotype on an average. The standard variety, Gull, has been crossed with all the others and some of these with one another. The material has been sown with marking boards in rows, 15 cm apart, with 15 seeds to the row and 10 cm between the seeds in the rows. Missing plants have been noted and plants next to empty spaces have not been included in the measurements, nor have the end plants in the rows. If the seed from the crosses has not been sufficient for a row, the row has been filled with spring wheat. In this way differences in the stand have been eliminated as far as possible. In most cases there have been far more than 15 seeds available of a combination. Replications have then been sown, using a plan similar to the one described for *Galeopsis* (HAGBERG, 1952 a) submitting, as far as technically possible, all biotypes to the variations in the soil.

The emergence of the plants has been annotated and some observations have been made in the early stages of development. Pollen fertility and seed setting have been determined and in certain F_1's the meiosis has been studied. The data of heading have been annotated and, finally, a number of weighings and measurements have been made on the ripe plants. The statistical treatment of the data has been made with the methods described in HAGBERG, *l. c.*

III. MAIN RESULTS OF THE COMPARISONS BETWEEN F_1 AND PARENTS.

1. SEED SETTING AFTER CROSSING. SIZE OF SEED AND GERMINATION.

The seeds obtained after artificial pollination of barley are usually less well filled and have a lower weight than normal seed, obtained after selfing without artificial manipulation. In 1947 the following values for the weight of 1000 seeds were obtained in Gull barley:

normal seed 44,5 g, artificially selfed seed 27,5 g. Obviously the latter are not able to offer the embryo and the young seedling as good a start as normal seed, and in order to obtain comparable material of F_1 and parents it is necessary to make artificial crosses also within the varieties.

It has been possible to make a direct, valid, comparison between crossing and selfing in regard to seed setting. A similar comparison in *Galeopsis* (HAGBERG, 1952 a) shows a tendency towards a better seed setting after crossing. In barley a similar tendency is absent or at least only weakly marked. There is an indication in the material of 1946, where there was an average difference in seed setting between crossing and selfing of $+10{,}05\ \% \pm 2{,}92$; $t = 3{,}44$, $0{,}01 > P > 0{,}001$. If the material of 1945, and especially that of 1947, is included in the calculation the difference is evened out and does not reach any level of significance.

The differences in this respect between *Galeopsis* and barley agree with the hypothesis offered to explain the superior seed setting after crossing in the former, *viz.* that the *Galeopsis* species only at a fairly recent stage of their phylogeny have become autogamous, and that remains of a system of *s*-alleles should still function. Barley, on the other hand, a very old domestic species, may be assumed to have been autogamous for a very long time, and there does not seem to be any remains of any *s*-alleles. In the case of the *erectoides* crosses, ert 19× ×Bonus and ert 21×Bonus (HAGBERG, 1953), it is probably the hybrid vigour which results in a higher degree of viability already in the earliest stages under the severe conditions caused by the manipulations of the flowers. The same explanation might be true in *Galeopsis*, but if such were the case there should be a correlation between the degree of heterosis in the individual F_1 and the superiority of seed setting after crossing in that special combination; such a correlation cannot be demonstrated, however.

A comparison between F_1 and parents as regards size of the seed does not show any difference, not even in the cases where heterosis has been demonstrated in other characters.

The germination has only been determined by the number of seedlings obtained in the field in percentage of the seeds sown; no differences were found between F_1 and parents. Both germinated rapidly and well, with the exception of 1947 when a severe spring drought caused a very uneven germination. Careful notes were made in this year, to use as a back-ground in judging the full grown plants, but not even in this case was there any difference between F_1 and parents. Nor was there any difference between reciprocal F_1's.

2. OBSERVATIONS ON YOUNG PLANTS IN 1946.

On two occasions, *viz.* four and seven weeks, respectively, after sowing the plants were studied and measurements were made. The results are presented in Table 1. On the first occasion the number of leaves on the first tiller was counted, the length of the second leaf blade on this tiller measured and the number of tillers per plant counted. At this stage F_1 is intermediate or closest to the parent line with the highest value.

After seven weeks the tillering was again determined and also the height of the highest — presumably the first — tiller. At this stage the number of tillers in most F_1's is as high or somewhat higher than in the best parent. In regard to the height of the shoot F_1 usually follows the most rapidly shooting parent. In one case, the combination

TABLE 1. *Measurements on the young plants of parental and F_1 material 1946.*

Combination	23/5			13/6	
	Number of tillers	Leaves on first tiller	Length of 2nd leaf	Number of tillers	Height of plant
K—A	1,8	3,9	99	9,2	22
K—A × K—B	1,9	3,9	106	9,7	26
K—B × K—A	1,8	3,9	106	9,4	25
K—B	1,7	4,0	109	8,8	25
K—A	1,5	3,9	97	8,0	17
K—A × K—C	1,7	4,0	95	9,7	17
K—C × K—A	1,7	3,8	94	10,2	17
K—C	1,6	4,0	90	10,7	16
K—A	1,8	3,9	98	8,8	23
K—A × K—D	2,0	4,2	97	9,8	28
K—D × K—A	2,5	4,6	97	9,8	28
K—D	2,5	4,7	95	9,0	27
K—A	1,6	4,0	103	10,1	21
K—A × K—E	1,9	4,0	99	8,9	24
K—E	2,2	4,1	96	10,5	32
K—E × K—F	1,9	4,2	105	6,8	26
K—F	1,6	3,8	123	8,6	30
K—A	1,5	3,8	99	8,3	17
K—G × K—A	2,3	4,2	103	8,9	21
K—G	2,2	4,2	101	7,8	20
K—A	1,4	3,8	102	8,5	18
K—A × K—K	1,6	3,9	101	7,8	22
K—K	1,7	4,0	102	6,9	27

K—E×K—F, F_1 shoots more slowly than both parents; the P-value for the difference is between 0,05 and 0,01. It is interesting that when ripe this F_1 is significantly taller than both parents.

On the whole, the lengthening of the straw during shooting takes place rather rapidly and the increase in height is not a good measure of the total growth of the plant, as it was in *Galeopsis*. On the other hand, the shooting is a measure of the rhythm of development: early or late.

3. EARLINESS: HEADING AND RIPENING.

The date of heading, fixed as the time when the whole of the ear is emerged from the sheath, is a fairly well defined stage in the development and, thus, a good measure of the rhythm of development. This date has been noted, the date of heading of a row being taken as the day when the ears had emerged on the main shoots of 50 % of the plants. A study of the data, a sample of which (the material of 1946) is presented in Table 2, shows that F_1, as a rule, is intermediate or as early as the earliest parent. In most cases the earliness is partially »dominant» (*cf.* the discussion of this term in HAGBERG, 1952 a). In 1948 F_1 of the cross K—N×K—L headed on an average 1,4 days before the earliest parent, but the difference is not significant.

The ripening has been studied, but it is difficult to obtain an exact estimate of the day of ripening. There are considerable differences in the material, especially between the parent lines, K—L and K—N being markedly earlier than, *e. g.*, K—A. As a rule, F_1 is intermediate or closest to the earliest of the parents.

TABLE 2. *The date of heading 1946. The first of July=1.*

Combination					
P_1	P_2	P_1	P_1+P_2	P_2+P_1	P_2
K—A	K—B	3,7	4,0	3,9	5,9
K—A	K—C	3,7	4,0	4,8	6,6
K—A	K—D	4,0	4,0	3,8	7,1
K—A	K—E	4,0	0,5		—1,5
K—E	K—F	—1,5	—0,5		—5,0
K—A	K—F	3,0		—1,0	—5,0
K—A	K—K	2,8	—1,0		—2,0
K—A	K—L	3,0	—8,0		—10,0
K—L	K—N	—10,0		—12,0	—14,0

4. MEASUREMENTS ON THE RIPE PLANTS.

After harvesting the plants, several weighings, measurements and other observations have been made on them. The data are presented in Table 3. The number of ripe ears per plant — final measure of tillering — is in most combinations the same in F_1 as in the best parent line. In 1946 the F_1's of the combinations K—A×K—G and K—A× ×K—D were significantly more tillered than the parents, but in the following years this superiority was not repeated. In no instance is F_1 inferior to the parent with the lowest number of tillers.

The height of the tallest straw, from the soil surface to the base of the ear, has been measured. As a rule, F_1 is intermediate, with the striking exception of the combinations between two-rowed and six-rowed varieties. In all the years when they were studied the F_1's of the crosses K—E×K—F and K--A×K—F were taller than the tallest of the parents, the differences reaching a high level of significance.

The length of the ear has been determined on the ear of the tallest straw. In this character F_1 is intermediate between the parents. Length of ear is positively correlated with »density of ear» if this latter character is defined as the length of 10 internodes; *cf.* HAGBERG, 1953. Laxity of the ear is partially dominant, as shown by NILSSON-EHLE (1939, 1948) in a number of crosses between mutants for density (*erectoides* mutants). On the whole the same holds true of length of ear. The length, however, is also dependent upon the number of florets per ear. This character has also been studied; in this case F_1 is again intermediate, and in no case has it been significantly superior to the best of the parents.

The grain yield of the plant is dependent upon several characters, some of which are already dealt with, such as tillering, number of florets per ear, or, rather, the number of those florets giving seed, and the weight of the seeds. On an average the grain yield of F_1 is equal to that of the best of the parents. In some combinations it is even superior to the best parent, but the differences do not reach any high levels of significance. F_1 of the combination K—A×K—D is superior to the best parent in all the three years, when it has been studied, but an analysis of variance gives a P-value for the difference of only 0,05—0,01. Also in the crosses between K—A and K—L and K—N the grain yield of F_1 surpasses the best parent, but with a very low level of significance. Finally, in the cross K—A×K—G F_1 is equal to the best parent in two years, in the third it has a superiority with a P-value of 0,01—0,001.

TABLE 3. *Data from measurements on the ripe plants. 1946—1948.*

Combination P_1 P_2	1946			1947			1948		
	P_1	F_1	P_2	P_1	F_1	P_2	P_1	F_1	P_2
	Weight of plant (grams)								
K—A×K—B	14,3	17,1	16,2	16,2	17,9	20,6	10,2	8,9	11,6
K—A×K—C	11,3	14,6	14,3	14,0	13,2	18,9	10,0	11,1	10,8
K—A×K—D	13,3	20,1	18,4	16,4	19,0	17,2	8,6	10,4	10,3
K—A×K—E	18,4	15,6	14,2	14,1	15,5	10,8	7,5	9,6	7,4
K—E×K—F	14,2	14,2	16,0	10,8	15,2	11,5	7,4	9,4	6,1
K—A×K—F				14,0	18,4	11,5	7,5	9,6	6,1
K—A×K—G	10,2	15,2	12,8	17,8	17,5	15,0	10,0	9,8	9,4
K—A×K—H				14,8	18,8	15,6	10,0	7,4	12,3
K—A×K—I							7,5	9,5	12,8
K—A×K—J				13,3	20,0	16,0	9,0	9,2	10,7
K—A×K—K	9,0	13,8	13,0	17,8	17,0	14,3	9,1	9,7	9,4
K—A×K—L				13,1	9,5	8,5	11,5	12,4	9,3
K—L×K—N							9,3	11,2	9,2
K—A×K—N				10,0	11,2	7,0	11,5	11,4	10,2
K—A×K—M							10,0	8,8	10,8
K—A×K—O							9,6	8,1	8,7
K—A×K—P							9,3	6,1	8,4
	Yield of grain per plant (grams)								
K—A×K—B	7,2	9,2	9,1	8,5	10,0	11,7	5,0	5,6	7,6
K—A×K—C	6,2	7,0	7,0	8,4	7,3	10,3	5,8	6,7	6,5
K—A×K—D	6,0	10,3	9,0	9,2	9,6	7,0	4,7	5,5	5,3
K—A×K—E				8,3	8,8	6,2	4,2	5,3	4,5
K—E×K—F	6,8	7,0	8,3	6,2	8,1	7,0	4,5	5,0	3,6
K—A×K—F				8,4	9,3	7,0	4,2	5,1	3,5
K—A×K—G	5,5	7,8	6,4	10,1	9,0	8,6	5,8	5,8	4,9
K—A×K—H				7,5	10,4	8,2	5,8	4,3	6,8
K—A×K—I							4,2	5,3	7,0
K—A×K—J				7,3	10,8	8,7	5,8	5,5	5,9
K—A×K—K	5,1	6,5	5,7	10,0	9,6	6,8	4,9	5,0	4,5
K—A×K—L				7,3	6,0	4,6	6,6	7,9	6,2
K—L×K—N							6,2	7,3	5,9
K—A×K—N				5,9	7,2	4,6	6,6	6,6	6,5
K—A×K—M							5,8	4,7	5,5
K—A×K—O							5,7	4,5	5,4
K—A×K—P							5,9	2,7	4,6
	Height of straw (cm)								
K—A×K—B	95	95	83	66	66	60	80	73	70
K—A×K—C	92	87	80	62	61	56	80	76	74
K—A×K—D	94	102	103	63	70	74	81	91	95
K—A×K—E				66	72	73	79	85	89

Combination P_1 P_2	1946			1947			1948		
	P_1	F_1	P_2	P_1	F_1	P_2	P_1	F_1	P_2
K—E×K—F	105	111	98	71	74	56	89	91	69
K—A×K—F				67	73	56	78	88	68
K—A×K—G	90	103	104	67	76	73	79	85	87
K—A×K—H				62	67	66	79	81	88
K—A×K—I							80	89	87
K—A×K—J				62	67	59	79	79	79
K—A×K—K	90	98	111	67	80	81	82	93	94
K—A×K—L				67	60	48	80	76	61
K—L×K—N							61	72	72
K—A×K—N				68	68	63	80	79	72
K—A×K—M							79	91	96
K—A×K—O							78	78	76
K—A×K—P							77	80	74
	Length of ear (cm)								
K—A×K—B	8,1	9,2	9,8	8,6	9,1	9,9	8,2	8,8	9,2
K—A×K—C	8,9	8,4	7,9	8,6	8,3	8,5	8,2	7,7	7,9
K—A×K—D	8,9	9,7	10,1	8,6	9,3	9,9	7,9	9,0	9,6
K—A×K—E				8,7	9,7	10,7	7,6	8,6	9,5
K—E×K—F	9,8	7,9	5,3	10,3	8,4	5,4	9,5	7,7	4,5
K—A×K—F				8,7	8,2	5,4	7,4	7,0	4,5
K—A×K—G	7,9	8,6	8,2	8,5	9,1	7,9	8,1	7,9	7,1
K—A×K—H				8,4	9,4	9,9	8,1	8,1	10,0
K—A×K—I							8,2	8,6	9,3
K—A×K—J				8,0	8,7	8,4	8,1	7,8	8,1
K—A×K—K	7,6	8,6	8,2	8,5	9,6	9,6	8,1	8,7	8,7
K—A×K—L				8,1	7,4	7,4	8,3	8,8	7,5
K—L×K—N							7,5	8,0	7,5
K—A×K—N				8,4	7,5	6,7	8,3	8,3	7,5
K—A×K—M							8,3	9,4	10,3
K—A×K—O							7,9	6,8	5,7
K—A×K—P							7,9	7,6	5,4
	Number of heads per plant — tillering								
K—A×K—B	7,7	8,4	8,2	7,6	7,8	8,9	4,1	3,8	4,0
K—A×K—C	6,4	8,1	8,4	7,4	6,5	9,2	4,0	5,1	5,1
K—A×K—D	7,2	8,3	6,8	8,1	6,9	5,6	3,9	3,9	3,3
K—A×K—E				7,2	7,0	6,1	3,3	3,8	3,4
K—E×K—F	7,0	5,6	6,1	5,5	5,5	4,0	3,4	3,4	2,4
K—A×K—F				7,6	6,7	4,0	3,4	3,4	2,3
K—A×K—G	5,8	6,7	5,3	8,6	6,5	5,4	4,1	3,5	3,2
K—A×K—H				7,5	7,6	5,8	4,1	2,9	4,1
K—A×K—I							3,4	3,1	4,1
K—A×K—J				6,0	8,4	7,8	4,2	4,0	4,0

Combination P_1 P_2	1946			1947			1948		
	P_1	F_1	P_2	P_1	F_1	P_2	P_1	F_1	P_2
K—A×K—K	5,9	6,3	5,4	8,6	7,0	5,4	4,0	3,5	3,2
K—A×K—L				6,5	5,2	4,6	4,7	5,2	4,7
K—L×K—N							4,7	4,8	4,2
K—A×K—N				5,3	6,1	4,1	4,7	4,7	4,6
K—A×K—M							4,1	2,8	3,2
K—A×K—O							4,5	2,9	2,5
K—A×K—P							4,8	1,7	2,1
	Thousand grain weight (grams)								
K—A×K—B	41	42	45	46	50	49			
K—A×K—C	41	42	42	46	46	45			
K—A×K—D				46	53	53			
K—A×K—E				46	47	45			
K—E×K—F	45	51	32	45	54	34			
K—A×K—F				46	54	34			
K—A×K—G	41	46	49	46	52	53			
K—A×K—H				46	54	54			
K—A×K—J				46	48	50			
K—A×K—K	41	43	46	46	51	53			
K—A×K—L				46	53	53			
K—L×K—N				53	56	54			
K—A×K—N				46	50	54			

	1948					
	Number of florets per ear			Seed setting %		
	P_1	F_1	P_2	P_1	F_1	P_2
K—A×K—B	24	24	26	97	98	98
K—A×K—C	24	22	22	97	98	98
K—A×K—D	23	23	25	96	96	95
K—A×K—E	24	24	24	97	97	97
K—E×K—F	24	24	(42)*	97	96	93
K—A×K—F	22	24	(41)*	97	97	93
K—A×K—G	23	25	24	97	98	98
K—A×K—H	23	23	25	97	98	97
K—A×K—I	24	26	26	97	98	98
K—A×K—J	24	24	23	97	99	96
K—A×K—K	23	21	22	97	96	95
K—A×K—L	24	22	19	97	96	95
K—L×K—N	19	21	19	95	96	93
K—A×K—N	24	22	20	97	97	93
K—A×K—M	23	24	24	97	97	98
K—A×K—O	23	19	(38)*	98	95	93
K—A×K—P	23	19	(35)*	99	96	91

* Six-rowed barley.

In regard to the total weight of the plants (except the roots) F_1 is superior to the best parent in many of the combinations tested. In the cross K—A×K—D this difference reaches a P-level of $< 0{,}001$ in 1946, in 1947 and 1948 the 0,05—0,01 level. In the cross K—A×K—G the superiority of F_1 over the best parent reaches the 0,01—0,001 level and, finally, it reaches the 0,05—0,01 level in the combination K—A×K—L, K—A×K—N and K—N×K—L.

5. FERTILITY.

The fertility has been studied as the percentage of morphologically good pollen in Belling glycerine preparations and also as the number of seeds in percentage of the number of florets. In some cases these observations have been supplemented by a study of the meiosis. The pollen fertility is high all through the material and the seed setting good, as seen from Tables 4 and 3, respectively. In 1946 and 1947 F_1

TABLE 4. *Percentage of good pollen in lines and F_1 combinations of barley.*

Combination P_1 P_2	1946			1947		
	P_1	F_1	P_2	P_1	F_1	P_2
K—A×K—B	94	97	98	96	96	94
K—A×K—C	94	96	95	96	92	97
K—A×K—D	94	98	98	96	97	96
K—A×K—E	94	93	95	96	97	98
K—A×K—F	94	93	91	96	92	96
K—E×K—F	95	93	91	98	93	96
K—A×K—G	94	91	92	96	88	96
K—A×K—H				96	98	95
K—A×K—J				96	97	96
K—A×K—K	94	96	98	96	97	96
K—A×K—L	94	93	93	96	98	93
K—L×K—N	93	93	85			
K—A×K—N				96	97	98

of the cross K—A×K—G gave a somewhat lower pollen fertility than the other crosses but the seed setting was nevertheless good. The meiosis was completely regular and in 1948 the pollen fertility also of this cross was quite normal. There are no sterility barriers in the material here studied as there were in the *Galeopsis* material (HAGBERG, 1952 a and b). Nor is the seed setting or the pollen fertility in F_1 better than in the parents, however (*cf.* BERNSTRÖM, 1950).

6. STUDIES OF F_1 COMBINATIONS OF AUTOTETRAPLOID BARLEY.

It is of great interest to compare diploids and the corresponding autotetraploids in regard to the occurrence of hybrid vigour. MÜNTZING *et al.* (1936, 1943) have produced autotetraploids of some barley varieties. Between three of these, Cowra (2-rowed from Australia), Ymer (cross-bred, 2-rowed, Svalöf), and Edda (cross-bred, 6-rowed, very early, Sweden), diallel crosses have been made in the diploid as well as in the autotetraploid stages. Comparisons have been made between F_1 and parents as in the previously described material and in regard to tillering, plant height, yield of grain, and total plant weight. Unfortunately the material, especially of the tetraploids, is too small

TABLE 5. *Plant height in parents and F_1 of crosses between diploids and between the corresponding autotetraploids.*

Biotype	Diploid			Tetra-ploid		
Cowra	94 cm			78 cm		
Ymer	95 »			79 »		
Edda	107 »			90 »		
		Relative value of F_1 when			Relative value of F_1 when	
		P_{max} = 100	P_{menn} = 100		P_{max} = 100	P_{mean} = 100
Cowra × Ymer	103 cm	108	108	78 cm	98	99
Cowra × Edda	105 »	97	104	90 »	100	107
Ymer × Edda	114 »	106	112	99 »	110	117

to allow any definite conclusions, but the data are of some interest and some of them are therefore published here. Tillering, yield of grain and plant weight are very strongly modifiable; so is the plant height, although this is somewhat less influenced by environment.

The mean plant heights of the different biotypes as well as the relative values for F_1 are summarized in Table 5. There are no definite indications of differences between diploids and tetraploids in regard to heterosis.

SOME WINTER WHEAT CROSSES.

Some studies of F_1 in winter crosses, made in collaboration with the Wheat Division of the Swedish Seed Association, may be briefly mentioned here. The crosses were made between one line from the cross-bred variety Skandia II and 30 differ-

ent strains of land-varieties from different parts of Sweden. The crosses were made in 1946 and the material sown in the fall of that year. The seeds obtained after artificial crossing were heavier and better filled than those obtained after normal selfing and the F_1 seeds sown were throughout heavier than the seeds of the parent lines, sown for comparison. As unfortunately no crosses within strains were made, it is impossible to know for sure whether this difference is due to heterosis only or also to the manipulations of the flowers giving F_1 seed. The effect of »artificial selfing» is investigated by GRIFFEE (1921) and by ENGLEDOW and PAL (1934). GRIFFEE found that the manipulation gave smaller grains while ENGLEDOW and PAL, however, found the opposite conditions. At the end of 40 days from sowing no differences were to be found in their material, however.

In the late fall the length of the second leaf of the main tiller was measured. In all crosses studied F_1 was superior to the best of the parents in this character. At the same time there was a marked correlation between the degree of this »heterosis» and the degree of differences in seed weight between F_1 and parents.

The following winter was very severe and was followed by an equally severe drought in 1947, so that only a comparatively small number of plants reached maturity. The stand was very uneven and the material could not be used for a closer analysis. The number of plants ripening in per cent of the number counted in fall is, on an average, higher in F_1 than in the hardiest parent; t for the difference is only 2,19*, however. There is no correlation between this difference and the difference in seed weight. In about one third of the combinations F_1 was hardier than both parents, in three crosses of the 30 studied it is definitely inferior to the best parent in this respect.

IV. INBREEDING DEPRESSION IN F_2—F_6 OF A SELF-FERTILIZER.

In the earlier part of this paper the occurrence and degree of heterosis has been judged by a comparison between F_1 and its parents. The same method was used in the studies on *Galeopsis* (HAGBERG, 1952 a and b) and is in accordance with the definition of heterosis as given by the originator of the term, SHULL (1914, 1948). The problem of whether heterosis occurs or not may also be attacked from another point of view, *viz.* by a study of the inbreeding curve, obtained by growing later generations of a cross. The present study of heterosis in barley was planned so as to give information from this point of view. In a recent paper on heterosis in maize, BRIEGER (1950) has pointed out certain difficulties, inherent in a definition of heterosis which is founded upon a comparison between parents and hybrids. He considers »heterosis to be present in those cases in which any quantitative character shows a higher mean in the hybrid generation than in any of the subsequent generations, obtained by any form of close inbreeding».

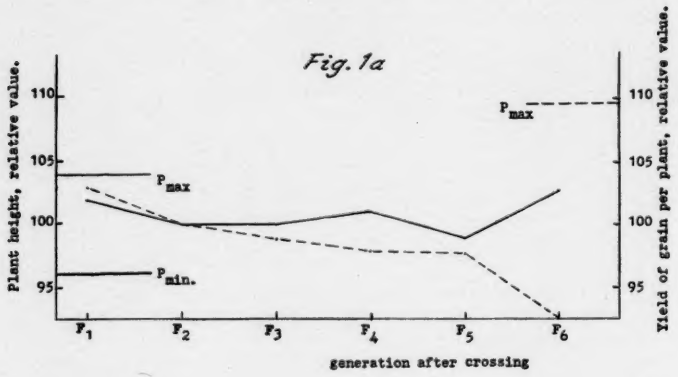


Fig. 1a
Plant height, relative value.
Yield of grain per plant, relative value.
110
105
100
95
P_{max}
$P_{min.}$
F_1 F_2 F_3 F_4 F_5 F_6
generation after crossing

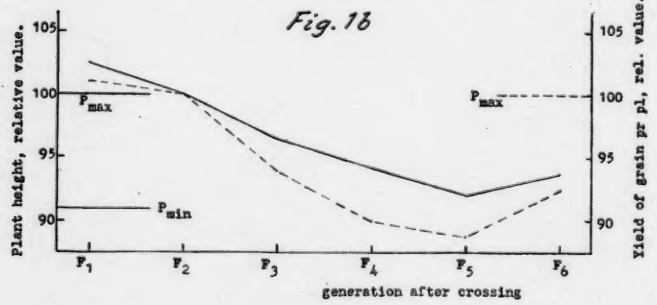


Fig. 1b
Plant height, relative value.
Yield of grain pr pl, rel. value.
105
100
95
90
P_{max}
P_{min}
F_1 F_2 F_3 F_4 F_5 F_6
generation after crossing

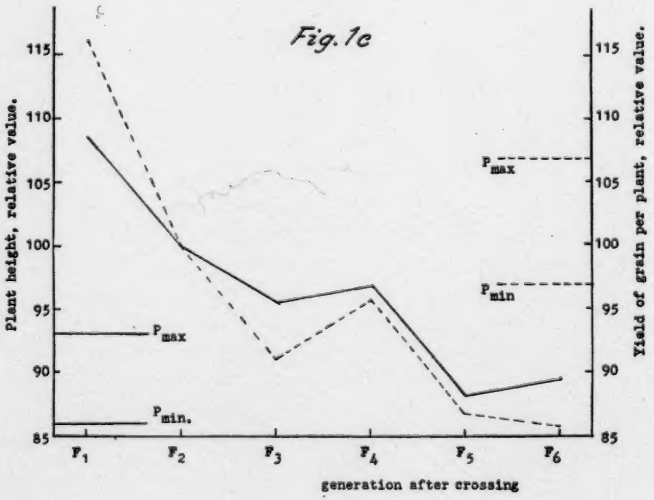
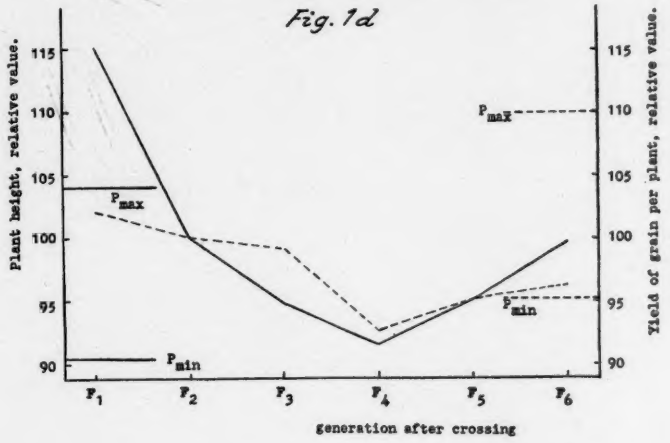


Fig. 1c
Plant height, relative value.
Yield of grain per plant, relative value.
115
110
105
100
95
90
85
P_{max}
P_{min}
P_{max}
$P_{min.}$
F_1 F_2 F_3 F_4 F_5 F_6
generation after crossing



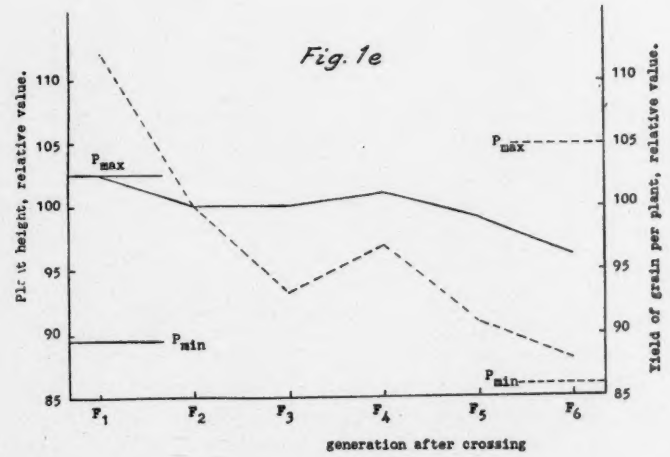


Fig. 1 *a*—*e*. Inbreeding curves for relative plant height (———) and yield of grain (- - - -); the six first generations after the crossings *a*. K—A×K—B, *b*. K—A×K—E, *c*. K—A×K—F, *d*. K—E×K—F, and *e*. K—A×K—G. The mean of the F_2 generation is given the value 100. The lines P_{max} and P_{min} give the value of both parental lines. In *a* and *b* P_{min} has a value lower than the lowest in the diagram and is left out of the figure.

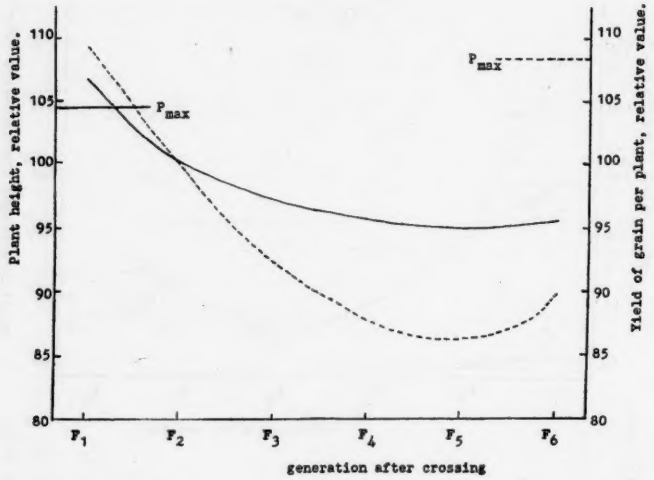


Fig. 2. Inbreeding curves for relative plant height (———) and yield of grain (- - - -). F_2=100. Mean value for the 5 cross-combinations replicated three years.

For technical reasons it has been impossible to study all the barley crosses made in F_1 to F_6; five different combinations were chosen for such a study. In 1947 it was possible to compare parents, F_1 and F_2, in 1948 also F_3 and, finally, in 1951 a comparison was made between the parents and all generations from F_1 to F_6. In a strict self-fertilizer as barley, F_2 may be considered as I_1, F_3 as I_2 *etc.*, and the parents are analogous to inbred strains.

The comparisons between the generations have been made in small variety tests with three replicates; the plots have been sown with marking board, each comprising 150 seeds sown. The results from different years have shown a very good agreement with one another. Fig. 1 shows the inbreeding curves obtained in 1951 for plant height and grain yield per plant, respectively, for each of the five combinations studied; the material of 1951 is chosen to illustrate the types of curves obtained, since it is the most complete one. Fig. 2 gives the corresponding curves for the average of all five combinations, the curves also include the data from 1950 and 1949. In the latter year the number of F_1 seeds available was not sufficient to allow a valid comparison with this generation. In all figures the scales used for the two characters are so determined as to allow a direct visual comparison

between them in regard to the percentual differences between the generations, the F_2 generation given the value 100.

In regard to plant height F_1 of the combinations with K—F, a six-rowed variety, is distinctly superior to both parents (cp. p. 331) and, as seen from Figs. 1 *c* and 1 *d*, the inbreeding curve for these combinations is more steep than those for the other combinations. The curve for the combination K—A×K—B shows only small differences between the generations, which in this case may be explained by the fairly close relationship between the parent varieties.

There is a tendency for the inbreeding curves for plant height here presented not to incline asymptotically towards a horizontal line, but rather to have a minimum somewhere from F_3 to F_5. The curve is most probably influenced not only by the inbreeding depression but also by a natural selection among the segregates, which is probably unequally strong in different combinations, depending upon the degree of difference between the parents and, thus, upon the degree of heterogeneity in the segregating material. Anyway, the result of the two forces in action is different in different combinations. K—A×K—B has only a weak depression and the minimum is possibly attained at about F_4, in K—E×K—F, with a strong depression, the minimum is nevertheless reached in F_4. In the other combination with a strong depression, K—A×K—F, there is an indication of a minimum in F_5, but it is rather weak, and the lowest value may possibly not be attained even in F_6.

The two characters, plant height and grain yield, for which curves are given are chosen to represent two different types of the characters for which such curves have been made, *viz.* the two mentioned ones, length of ear, total weight of plant, *etc.* In regard to yield of grain the selection has been towards high values (and of the five strains included in the five combinations at least the four are the result of a systematic selection); in regard to the height of plant, on the other hand, the breeder, searching for a stiff straw, has on the whole selected towards low values. In regard to the latter character there occurs a heterosis, according to the definition of SHULL (*l. c.*) in at least two cases, while the phenomenon is less marked in regard to grain yield. If the degree of inbreeding degeneration, according to BRIEGER (*l. c.*), is accepted as a measure of heterosis, there is no marked difference between the two characters.

In regard to yield of grain the strains must be expected to have values approaching the F_1 value or at least being considerably above the mean of the inbred strains obtained from the cross between them;

this mean is represented by the asymptote or, where there is a marked minimum, by a value more or less below this minimum. In regard to plant height, on the other hand, the parent strains may be expected to have values near to or even below the lowest value of the inbreeding.

V. DISCUSSION.

The difficulty to obtain a sufficient number of F_1 seeds for a comparison between parents and F_1 is most likely the reason why there are only rather few studies of heterosis in cultivated, self-fertilizing species, such as wheat, oats and barley. There are, however, a few papers on heterosis in wheat (*e. g.*, GRIFFEE, 1921; ROSENQUIST, 1931; ENGLEDOW and PAL, 1934; BOYCE, 1948) and oats (*e. g.*, COFFMAN and DAVIES, 1934; COFFMAN and WIEBE, 1930). Barley was studied by ENGLEDOW and PAL (1934), who did not find any marked differences between parents and F_1. IMMER (1941) found significant superiority in yield of grain for F_1 in one out of six crosses studied. SUNESON and RIDDLE (1944), using a gene for male sterility, have been able to obtain a large F_1 material of barley without the manipulations necessary for emasculation and pollination, and they have obtained sufficient material for a valid comparison between F_1 and parents. On an average they find that F_1 is about 16 % higher than the mean of the parents in yield of grain, but F_1 is superior to the best of the parents only in three out of seven combinations studied.

ENGLEDOW and PAL (*l. c.*) do not state definitely whether they have used emasculation and artificial pollination in obtaining the seed of the parents used in the comparisons between these and F_1 in barley. If they have not taken this precaution the F_1 seed of barley has been inferior in quality to that of the parents; in wheat the relation has been the opposite one (cp. p. 337). ENGLEDOW and PAL (*l. c.*) have also studied the effect of artificial selfing of six wheat varieties. The artificially selfed seed germinated more rapidly than seed from normal selfings, but after 40 days there was no evident difference between the plants from the two types of seed. GRIFFEE (1921) and ROSENQUIST (1931) have used artificially selfed parent material in their studies on wheat.

Several studies on barley have more or less directly treated the occurrence of monofactorial heterosis (TEDIN and TEDIN, 1926; ROBERTSON *et al.*, 1932, 1935; GUSTAFSSON, 1946, 1947, *etc.*; NYBOM,

1950). The present author has discussed these investigations in another publication (*cf.* HAGBERG, 1953).

As seen from this brief review, there are only rather few reports on the occurrence of heterosis in crosses between different varieties or strains of barley. — The first part of the present paper deals with a comparison between F_1 and the parents. As was the case in *Galeopsis*, F_1 is usually most similar to the most rapidly growing parent. There are exceptions, however, *e. g.*, F_1 of the cross K—E×K—F, which six to seven weeks after sowing is inferior to both parents in plant height and also in tillering. At ripening the same F_1 has a significantly taller straw than either parent; its yield is equal to that of the best parent but the tillering is still weak.

For a detailed analysis of heterosis it is important to resolve complex characters as yield of grain into components, such as tillering, number of kernels per ear, weight of seed, *etc.* (*cf.* IMMER, 1941). As pointed out by POWERS (1944) and BOYCE (1948), it is then often found that heterosis in, *e. g.*, grain yield depends upon a combination of favourable dominance in the component characters: »combination heterosis» (HAGBERG, 1952 b). Morphologically such an analysis of complex characters may be carried very far; from a genetical point of view it would have been desirable to be able to follow it down to the effect of single genes. A character such as length of ear may primarily be divided into number and length of internodes. The latter character is influenced by a number of genes, which it has been possible to study in some detail because of the high frequency with which they mutate after X-ray treatment (*erectoides* genes). The effects of the individual *erectoides* genes and their interactions are discussed by HAGBERG (1953).

In barley heterosis is found mainly in characters in regard to which man has made no selection or a negative one. Such a character is the height of the plant.

It is of interest not only to compare F_1 with the parents but also to study the shape of the inbreeding curve. Some investigators (COFFMAN and WIEBE, 1930; ENGLEDOW and PAL, 1934; IMMER, 1941; BOYCE, 1948) have compared F_1 with F_2 and F_3 in some self-fertilizing species, but they have made no comparisons between the inbreeding curves for different characters or combinations.

As mentioned, BRIEGER (1950) takes only the inbreeding curve into consideration when estimating the degree of heterosis, not the relation between F_1 and parents. The present data are well suited to

illustrate the difference between the two concepts of »heterosis». Having, more or less consciously, selected for high values of a certain character, *e. g.* yield, heterosis is rarely found as a superiority of F_1 over the parents; such heterosis is rather common, in characters such as plant height, in regard to which man has made a negative selection or none at all. If F_1 is compared with the following generations after selfing, however, the inbreeding curves for both characters are rather similar. According to BRIEGER (*l. c.*), the degree of heterosis should be the same in both characters. In the author's opinion, however, the term heterosis should be reserved for the phenomenon, *viz.* a superiority of F_1 over the parents, for which it was originally introduced by SHULL (1914, 1948). On the other hand, differences in the degree of inbreeding degeneration in latter generations of a cross are obviously of the greatest interest and important for the interpretation of the phenomena involved.

Finally, the results of the present investigation may be compared with the ones obtained in the author's study of heterosis in *Galeopsis* (HAGBERG, 1952 a and b) and with the conclusions drawn from the latter. Heterosis occurs in crosses between cultivated varieties of barley but it is less marked than in *Galeopsis*, especially in the allotetraploid species of this genus. The frequency of crosses showing heterosis is lower in barley, and the degree of heterosis, when occurring, is less than in *Galeopsis*. No difference in respect to heterosis has been found between diploid and autotetraploid barley in the somewhat scant material studied. Nor is there any relation between the degree of heterosis and the geographical differentiation between the parents in the present material, which is rather limited in extent. By the interference of man the distribution of barley has been rather efficient, and the exchange of material from one region to another has been rather intensive, especially in more recent times. It is, therefore, far from certain that the »land varieties» from which several of the strains here used have been selected, have been for any considerable length of time established in the region where they have been collected, nor that they are typical of these regions.

No sterility barriers have been found within the material, not even in crosses between the two »species» *Hordeum vulgare* and *H. distichum*, and it has not been possible to study the relation between degree of heterosis and degree of reduction in fertility. Heterosis is more marked in crosses between *vulgare* and *distichum* than in crosses within the latter, which may indicate some degree of genotypical differentia-

tion between the groups, justifying a taxonomical distinction between them as sub-units within a species.

The variability between plants is not greater in F_1 than in the parent lines. The number of combinations studied is too small (17) to allow a valid comparison between the variation between F_1's and that between parent strains, and it is not possible to demonstrate the occurrence of two types of »dominance» as found in *Galeopsis* (HAGBERG, 1952 b).

SUMMARY.

In 17 different combinations between barley strains F_1 has been compared with the respective parent strains. Artificial pollination after emasculation has been made within the strains as well as for the crosses.

There is hardly any tendency towards a better seed setting after crossing than after selfing. The weight of the kernels is about the same after crossing as after selfing.

No differences between F_1 and parents have been found during germination. Some measurements and other observations have been made on the material four and seven weeks after sowing. As a rule, F_1 is intermediate or close to the parent with the most rapid development. The same holds true for heading and ripening: F_1 is usually intermediate or equal to the earliest of the parents.

A number of different characters have been studied on the ripe plant. In weight of the whole plant F_1 is often superior to the best parent or at least as heavy as this one; in some combinations F_1 is intermediate, however. In regard to tillering, length of ear, weight of 1.000 seeds and yield of grain per plant most of the F_1 combinations are intermediate or equal to the best parent. In F_1 of crosses between two-rowed and six-rowed barley the weight of 1.000 seed is about 20 % superior to the two-rowed parent, which has heavier seed than the six-rowed one.

Fertility as well as meiosis is completely normal in all the combinations studied.

Three combinations have been made between three autotetraploid strains as well as between the corresponding diploids. No difference has been found between diplo- and tetraploids in regard to the degree of heterosis.

In five of the combinations F_1 has also been compared with the following generations, F_2—F_6. In this way inbreeding curves have been

obtained and their shape as well as their position in relation to the parental values has been studied. In this connection the opinion of BRIEGER (1950) is discussed, *viz.* that the degree of heterosis should be measured by comparing F_1 with the following generations, disregarding the values of the parents.

A comparison between the inbreeding curves for different characters shows that the shape of the curve as well as its position in relation to the parental values is dependent upon the principles governing the selectional processes by which the parent strains have been more or less consciously chosen by man. Heterosis, as defined by SHULL (1914, 1948), is found mainly in characters where the breeder either has not selected at all or has selected in a negative direction, *e. g.*, height of plant. In characters, *e. g.*, yield of grain, where the selection has been positive, such heterosis is absent to weak. If BRIEGER's definition of heterosis is accepted there is no difference between the two types of characters in regard to the occurrence or degree of heterosis.

In many cases the inbreeding curves do not approach asymptotically to a horizontal line but have a marked minimum in F_3—F_5. This is probably due to a process of natural selection, eliminating the poorest types and thus, to some extent, counteracting the inbreeding degeneration.

Svalöf, in June 1952.

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FURTHER STUDIES ON AND DISCUSSION OF THE HETEROSIS PHENOMENON

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I. INTRODUCTION.

THE heterosis problem has a central position in present day genetics and it has connections with plant and animal breeding and also with the study of human populations. A deeper knowledge of the heterosis phenomenon has made it possible to utilize heterosis successfully in practical breeding work. The breeding of hybrid corn is the outstanding example; F_1 seed of sugar beet is also of a considerable importance, *etc.*

The results already obtained have stimulated further research into the processes behind the phenomenon of heterosis. A large number of scientists have attacked the problem, experimentally and theoretically, and there is a vast literature on heterosis. A special conference on the subject was held at Ames, Iowa, U.S.A., in 1950 and the Proceedings of this conference (GOWEN *et al.*, 1952) give a comprehensive review of the present state of the research on heterosis.

The Proceedings start with three papers by ZIRKLE, SHULL and HAYES which present a detailed history of the research on hybrids and the development of the concept of heterosis. The present author, therefore, desists from any historical review and refers to the papers mentioned. Nor does the author intend to attempt a comprehensive survey of the whole problem. He will restrict himself to presenting a summary of his own investigations on heterosis, discussing their results and making an attempt to connect these with the results of other investigators.

In 1945 the author started a series of experiments with different materials in an attempt to study some aspects of the phenomenon of heterosis. The results have been published in a series of papers (HAGBERG, 1948, 1952 a, b and c, 1953 a and b). At the start the following part problems were included in the program:

(1) Which definition of heterosis is to be preferred and what is the correct method of measuring heterosis?

(2) When and where does heterosis occur? In which types of

crosses? Does it occur in self-fertilizing species as well as in cross-fertilizing ones?

(3) Is heterosis equally marked in diploids and in tetraploids?

(4) Is there any relation between the degree of heterosis in F_1 and the degree of ecological or geographical differentiation between the parents?

(5) Is there any relation between the degree of an occurring reduction in fertility and the degree of heterosis in F_1?

(6) In what way does heterosis manifest itself? During which stages in the ontogenesis is it found?

(7) Is F_1 of a cross between two pure lines more variable than the parents and, if such is the case, is there any relation between the degree of variability and that of heterosis?

(8) Does superdominance occur? Which theory offers the best explanation of the observed cases of heterosis?

In order to contribute to an elucidation of these problems crosses have been made in four genera of essentially different biological types. Two were cross-fertilizers, *viz.* red clover and rye, two were self-fertilizers, *viz. Galeopsis* and barley. The red clover material used consisted of local strains, cultivated but differentiated by natural selection, not by systematic breeding. The rye strains used were high-bred varieties, the result of strict selection by plant breeders. *Galeopsis* is a wild growing plant, but at least three of the four species here used are strongly dependent on man for their distribution. The material of barley, finally, consisted of cultivated varieties, some of which are the result of intense breeding, some of which, however, are lines from »land-varieties». In the self-fertilizing plants — *Galeopsis* and barley — crosses between diploid lines were studied and compared with crosses between lines of allotetraploids and between lines of autotetraploids, respectively.

The crosses in red clover and rye have been studied in regard to a few characters in order to ascertain the occurrence of heterosis. No detailed comparisons between F_1 and the parents have been made (HAGBERG, 1952 c). In the material of *Galeopsis*, F_1 has been compared in detail with the parents at different ontogenetical stages; the studies on this material have concerned most (2)—(7) of the problems listed above (HAGBERG, 1952 a and b).

In barley F_1 has been compared not only with the parents but with the following generations after selfing, *i. e.* the effect of inbreeding after a cross between two homozygous lines of a self-fertilizer has been

studied (HAGBERG, 1953 b). Finally, X-ray mutations in barley have been used for a study of dominance and superdominance in monofactorial hybrids (HAGBERG, 1953 a).

In the papers mentioned the original problems have already been discussed in some detail. The author has tried to avoid repetitions as far as possible and the disposition of the material in this paper does not follow the original program absolutely strictly.

II. THE HETEROSIS CONCEPT.

There is an old saying that »a beloved child has many names». In the case of heterosis a beloved name or, rather, a useful term has on the opposite been used in many different senses — to cover rather different concepts. The present author has discussed the problem of terminology at some length in the introduction to one of the previous papers (HAGBERG, 1952 a). It may be suitable here again to give a brief summary of the opinion of the author.

The salient fact is that it was SHULL who coined the term heterosis in 1914 and gave its definition. In some respects the definition is a little vague but nevertheless the term is comprehensive and useful. It may without difficulty be retained as a comprehensive term for the phenomenon that there is often an increase in size or in other valuable qualities in the cross-bred as compared with the pure biotypes, the different varieties or, even, the different species whose union produced the cross-bred in question.

The terms heterosis and hybrid vigour are widely used as synonyms and it seems evident that SHULL (1914) considered them to be so when he introduced the former term. WHALEY (1944) and several other authors are of the opinion that an interpretation of the phenomenon according to a »stimulance theory» was inherent in the term heterosis, but SHULL (1948) repudiates this inference.

Recently, WHALEY (1952) has differentiated in another way between heterosis and hybrid vigour. According to him, hybrid vigour should denote the end result, the phenotypical superiority, while heterosis should be restricted to mean the mechanism which results in hybrid vigour. It would be valuable to keep this distinction and have a specific term for the new concept. The attempt to give a new meaning to the old and much-used term heterosis, which does not agree with SHULL's original definition, seems doomed to be a failure, however.

GUSTAFSSON (1952) has classified the cases of heterosis into three groups: (1) somatic heterosis, (2) reproductive heterosis, and (3) adapt-

ive heterosis. The distinction is useful and valuable but, as pointed out by GUSTAFSSON himself, the three types are not absolutely independent. Somatic heterosis in certain characters may easily result also in reproductive heterosis, *etc.*

Plant breeders are mainly interested in heterosis of the types (1) and (2) and the third type has, perhaps, been overlooked in many investigations. In many cases it is difficult, even impossible, to determine and in other cases it has had no direct interest for the tasks at hand. It is, therefore, of great value that population geneticists have stressed the importance of adaptive heterosis and taken it under discussion — but there is no need to exaggerate its importance at the cost of the others.

The author's experiments have been concerned with heterosis of the types (1) and (2). Mainly F_1 has been compared with the parents but in some cases also with F_2 and the following generations. Which of these comparisons is the most adequate one in studying heterosis, and, in comparing F_1 and the parents, should the superior parent be used or a mean between the parents?

In many cases a comparison between F_1 and the following generations will offer valuable information about the mechanism of heterosis, as discussed in detail by the present author (HAGBERG, 1953 b). This fact, however, does not justify a deviation from the original definition of SHULL, which necessitates a comparison with the parents. Here again the superior parent should be used as »standard», not a mean between the parents. There are different kinds of means, arithmetic, geometric, *etc.* and in all instances the mean is dependent upon the scale used. Thus, not only is the use of a mean against the original definition, it is not even unequivocal (*cf.* MATHER, 1946).

III. THE OCCURRENCE OF HETEROSIS.

This section deals with the original questions (2)—(5).

A. THE OCCURRENCE OF HETEROSIS IN RELATION TO DIFFERENT MODES OF FERTILIZATION.

The mode of fertilization is, evidently, of fundamental importance for the development of the mechanism resulting in heterosis. Different opinions have been expressed as to the relation between mode of fertilization and heterosis. According to some authors (ENGLEDOW and PAL, 1934; *cf.* also DARLINGTON and MATHER, 1949), heterosis is not to be expected among self-fertilizers. Nor should crosses between

populations of cross-fertilizers result in heterosis (*cf.* DOBZHANSKY, 1950).

If at first we turn to the results obtained by the present author in crosses in allogamous species (HAGBERG, 1952 c) we will see that heterosis occurred in crosses between rye populations which had been kept separated. No heterosis was found, however, in crosses between some local strains of red clover. The variation within these strains is very great, and not at least so in physiological characters, as shown by the rapidity with which they change under altered environments (NILSSON-LEISSNER, 1948). Even if the strains are morphologically fairly distinct the relative increase in genetical variation upon crossing two populations is probably comparatively small. With the current theory concerning the ultimate cause of most heterosis phenomena this would explain why no such phenomena were observed in this material.

The parental populations of rye used in the experiments have been considerably more differentiated. They are the result of systematic breeding towards certain desired characters in the course of which a fairly strict family breeding has resulted in a marked decrease in the genetic variation within the populations. The so-called »land-variety» from Od is not a true land-variety, but a family bred from such a variety. It has been bred with strong selection and family breeding for several generations, just as the Petkus rye used, and the two populations have been held strictly separated. The cross between them shows a definite heterosis. The population obtained under the name of »Giant» is of unknown origin, but probably it is only a Petkus, grown for some generations without selection or isolation. The two populations Petkus and Giant should then be fairly closely related — and their cross does not show the same marked heterosis as did the crosses Petkus × Od and Giant × Od.

From the practical breeding work with several different allogamous species it is known that the crossing of different, more or less uniform or »narrow» populations results in an increase in vegetative production (*e. g.*, root yield in sugar beets; RASMUSSON, 1951) or in reproductive capacity (*e. g.*, in rye; *cf.* HAGBERG, 1952 c). Heterosis does occur not only in crosses between inbred strains of cross-fertilizers as maize and rye (MÜNTZING, 1943 a) but also in crosses between populations, if these are fairly uniform and reasonably different genetically.

Heterosis is found also among autogamous species. The phenomenon has been clearly shown to occur in crosses in the 32-chromosome species of *Galeopsis*, subgenus *Tetrahit* as well as in *Hordeum*

crosses (HAGBERG, 1952 a, b, 1953 a, b). These results are in accordance with the experience from several other self-fertilizers as wheat, beans, oats, *etc.* (*cf.* HAGBERG, 1952 a, 1953 b), and there is no doubt that heterosis may be found also in autogamous plants. As pointed out (HAGBERG, 1953 b), there is, however, a considerable difference in degree between the heterosis observed in *Galeopsis* and that in barley, a fact that will be further discussed in a later section of this paper.

B. COMPARISON BETWEEN WILD AND CULTIVATED SPECIES.

Both the allogamous species studied are cultivated, but as already mentioned the strains of red clover are very much less influenced by man than are the varieties of rye used. Of the two autogamous species studied one is definitely wild, the other cultivated. A very marked heterosis occurs in many combinations within the wild *Galeopsis* species but it is considerably more difficult to prove heterosis in the cultivated barley. The difference may be due to several different causational factors.

The strict selection towards high yield which is the ultimate aim of the breeding work is one such factor. In using »high-bred» varieties of cultivated species we are dealing with biotypes which are not representative of the species they belong to but which are extreme plus variations in certain characters, desirable to the breeder. The selection in wild self-fertilizing species is not as one-sided or as consistent as the selection made by the breeder.

In such a character as yield of grain the F_1 in barley is often equal to the parents but the following generations show a more or less rapid decrease upon inbreeding (HAGBERG, 1953 b). In regard to another character, *viz.* plant height, there has been no similar selection towards high values — in many cases the selection has been negative for a fairly long period of years. In this character F_1 is superior to the parents in several combinations. Nevertheless, the inbreeding curve is about the same as in the case of yield of grain.

In cases of this type it is near at hand to measure the degree of heterosis by the difference between F_1 and F_2 or by some other characteristic of the inbreeding curve. According to BRIEGER (1950), this should be the only correct way to measure heterosis. This mode of measuring cannot be used in all cases, however. It is hardly applicable, for example, in the case of structural heterozygotes, such as found in *Oenothera* and *Paeonia* or in DOBZHANSKY's inversion heterozygotes

of *Drosophila pseudoobscura*. Then, again, it is not in accordance with the definition of heterosis. On the other hand, it may be once more pointed out that the inbreeding curve may offer valuable information regarding the genetic constitution of F_1 and, thus, as to the genetic differentiation between the parents, and in this way further light may be thrown on the problem of heterosis.

There is no fundamental difference between wild and cultivated species in regard to the occurrence of heterosis, but the artificial selection — often very strict and one-sided — which has resulted in the »high-bred» varieties of cultivated self-fertilizers may explain why heterosis is rarely found in crosses within such species. It is true that DOBZHANSKY (1952) states that luxuriance appears to be more frequently encountered in domesticated than in wild species, but so far as the present author knows, no proof for this opinion is found in the literature.

There is, as already mentioned, a further important difference between the materials of barley and *Galeopsis*. The tetraploid species of the latter genus, *viz*. *Tetrahit* and *bifida*, are allotetraploids and the diploid parental species are mainly allogamous (*cf*. HAGBERG, 1952 b). As already pointed out (*l. c.*), the autogamy of the allotetraploid species is probably acquired rather late during their phylogeny (*cf*. DARLINGTON and MATHER, 1949). Therefore, it seems probable that the genes of *Galeopsis*, with their reactions and interactions, have been selected mainly during a state of allogamy. And this, again, may explain why a marked heterosis is so often encountered in crosses between homozygous lines of the autogamous, allotetraploid species of this genus. The homozygous lines are closely comparable to inbred lines from allogamous populations.

Also in barley the greater part of the genotype has probably been selected during a state of allogamy. Most likely, however, the autogamous period has been longer in the phylogeny of barley than in that of the *Galeopsis* species studied. The gradual accumulation of new mutations — aided by the more or less one-sided selection occurring under cultivation — has gradually changed the genotype so as to include numerous genes which in their reactions and interactions are indifferent to heterozygosity.

The inferences just made include a hypothesis, according to which there is an essential difference between allogamy and autogamy in evolving genotypes which react with heterosis upon crossing. With allogamy genes will be selected whose effects and interactions are such

as to result in heterosis. (This assumption clearly includes a postulate that the somatic heterosis caused by these genes will also be an adaptive heterosis, but as will be discussed later on such a postulate is by no means unlikely.) Autogamy, on the other hand, would tend to favour genes whose reactions and interactions are most beneficient to the plant when they are homozygous.

There are probably no absolutely autogamous plants, the whole genotype of which has been selected and evolved under autogamy — or, in other words, in all higher organisms at least some part of the genotype has been selected during a state of allogamy. The longer the period of autogamy, however, the further the process of selecting new mutations indifferent to heterozygosity may have advanced. This process is very slow, however, and the major part of the genes will remain unaffected. This hypothesis offers a possible explanation of the different degrees of heterosis found in crosses in *Galeopsis* and in *Hordeum*.

There is still another difference between the two groups of material, and this will be discussed in the next section.

C. DIPLOIDY AND POLYPLOIDY.

As mentioned above, the genus *Galeopsis* has been represented in these studies by pure lines of diploid as well as of allotetraploid species. So far we have only dealt with the latter and compared them with the diploid barley. What occurs in tetraploid barley, and is there an indication of a general difference between diploids and tetraploids as regards the occurrence of heterosis?

Besides between diploid lines of barley crosses have also been made between three artificial autotetraploids (HAGBERG, 1953 b). There is no difference between these tetraploid crosses and the corresponding ones between the mother diploids in regard to the occurrence or degree of heterosis in F_1. Only a few crosses were made within or between the diploid species of *Galeopsis*, but they strongly indicate that heterosis is, on an average, more marked in the allotetraploids than in the diploids. The series in barley and in *Galeopsis* are not directly comparable and the material is rather scant. Nevertheless, there seems to be a clear indication that allotetraploids, as those in *Galeopsis*, have another relation to diploids with regard to the occurrence of heterosis than have autotetraploids, for instance, those in barley. The number of different genes in a strict autotetraploid is not larger than in the corresponding diploid, whereas the allotetraploids have a larger number of genes and hybrids between them will, on an average, have a higher

degree of heterozygosity than hybrids between the diploids; there are more loci in which heterozygosity may occur.

EAST (1936) discusses the difference between the auto- and allotetraploidy. He approaches the problem from quite a different point of view, however, and does not deal with experimental comparisons between crosses within allo- and autotetraploids in relation to crosses within the corresponding diploids. He points, instead, to the great competitive ability and wide distribution of the allopolyploids. There is no doubt that this conclusions contain part of the truth, but his arguments have been severely criticized, among others by MÜNTZING (1945 b).

As seen from the map of distribution (Fig. 1), the allotetraploid *Galeopsis Tetrahit* has a considerably wider geographical range of distribution than the two diploid species from which it has been synthesized in nature. This is only one among they very numerous instances of a superiority of the allopolyploids in the respects mentioned.

G. Tetrahit is autogamous and is made up of a number of homozygous lines. In regard to each of the two genomes involved this ought to result in inbreeding and decreased vigour. On the other hand stands the interaction between the genes from different genomes which has resulted in hybrid vigour and which is preserved — when the combinations have been favourable — by the allopolyploidy. This heterosis — on the diploid level — is of the somatic type according to the classification of GUSTAFSSON — luxuriance, according to DOBZHANSKY — but it has has evidently given the species hybrid — on the allotetraploid level — a considerably increased selective value. Possibly, the *gigas* characters, caused among other things by an increase in the cell-size, and which according to MÜNTZING and others are typical of the polyploids, have also contributed to this increase in selective value. In so far as the somatic heterosis has contributed it belongs also to GUSTAFSSON's adaptive heterosis or to the euheterosis of DOBZHANSKY. The case seems to offer a good example of the fact that a »hybrid luxuriance» which primarily is of a somatic type may also very well be adaptive and thus to be counted as »euheterosis».

The difference between allo- and autotetraploids is not distinct, however. According to MÜNTZING (1936), an amphidiploid or an allotetraploid may be a partial autotetraploid. KIHARA and ONO (1927) define an autopolyploid as formed by a reduplication of the same genome, an allotetraploid by the addition of two different genomes. The distinction, thus, depends upon whether the existing differences between two chromosome sets are considered to be sufficient to charact-

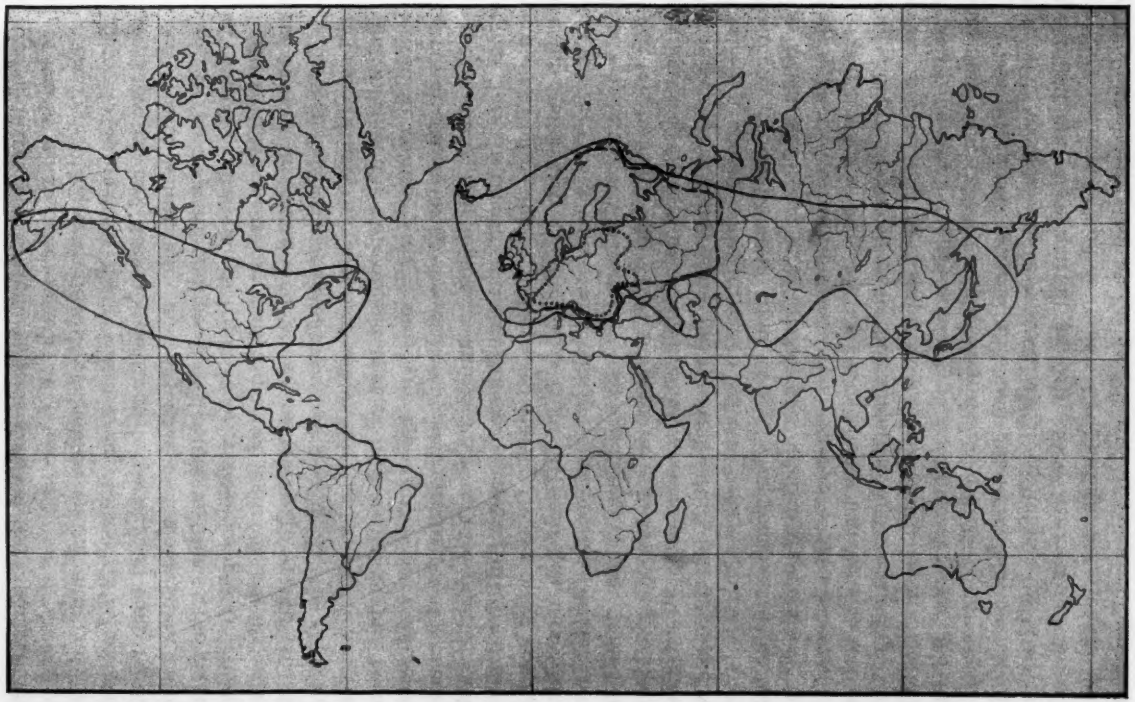

Fig. 1. A map showing the distribution of the species *Galeopsis Tetrahit* ——, *G. speciosa* - - - -, and *G. pubescens* . . . . ., according to informations obtained from Prof. MEUSEL, Halle, Germany.

erize these as different genomes or only as two variations of the same genome. If absolute identity between all four sets were required, autotetraploids could be formed only by doubling the chromosomes of pure lines. It is, therefore, not unreasonable to turn MÜNTZING's statement around and state that most spontaneous and induced autotetraploids are partial allotetraploids (*cf.* also STEBBINS, 1950).

Very marked heterozygosity is often found in hybrids within a diploid species and it is not rare to find heterozygosity also for structural changes of the chromosomes with a reduced fertility in the hybrid as a result. Some of these cases of structural heterozygosity will be discussed in the next section. Such sterility barriers within a species are often considered to indicate a beginning differentiation into new species. If and when such a barrier is definitely to be considered as a species barrier is mainly a question of definitions.

If the chromosome number of such an intraspecific hybrid is doubled the product is an autotetraploid according to the definition. On the other hand, since the two sets were at least partially distinctly different, the tetraploid also retains a high degree of »heterozygosity». In this case, namely, the alleles situated in the structurally changed or different segments do not, as a rule, behave as alleles during meiosis. Such a case leads over to the allopolyploids. The interaction between genes from different genomes may in many instances be of the same nature as the »heterozygosity» preserved in the above described type of autotetraploids. Due to the extent of the structural and genetical differentiation between the parental genomes the frequency of tetravalents at meiosis is higher in the autotetraploids than in the allotetraploids, however, and the former will retain heterozygosity to a lesser degree than the latter.

An autotetraploid of a cross-fertilizer as rye is always more or less of an allopolyploid. It is subject to one or the other type of selection which has a chance to result in complete heterozygosity in many loci, *i. e.* the four homologous chromosomes will all have different alleles, $A_1A_2A_3A_4$. The chances are therefore considerable to obtain, after some generations of selection, an increasingly good adaptation of a population of an allogamous autotetraploid, provided the number of genes is sufficient, *i. e.* that a sufficiently large portion of the genetic variation of the diploid has been carried into the tetraploid population.

A doubling of the chromosomes of a homozygous diploid self-fertilizer gives, on the other hand, a strict autotetraploid, with four identical genomes. Only in such material it is possible to study the

effect of chromosome doubling without the interference of heterozygosity and possible heterosis. The strict autotetraploids in barley and in flax are weak, without any practical interest so far, and completely unable to compete with the diploids. There is no genetic variation until a sufficient number of mutations have occurred, and until then all selection will be without any effect. Breeding of autogamous diploids by chromosome doubling should start from hybrids with the highest possible degree of heterozygosity. In this way there is a possibility for selection within the tetraploid, a possibility which will increase still further after crosses between different tetraploids. In this connection compare MÜNTZING's (1943 b) work with tetraploid barley and LEVAN's (1948) with tetraploid flax.

D. STRUCTURAL HETEROZYGOSITY.

In several different organisms heterosis has been observed in connection with structural heterozygosity. MÜNTZING (1945 a) and later HAGBERG (1948, 1952 b) could show that a relation exists between the degree of heterosis in F_1 and the degree of the reduction in fertility. The present author (1952 b) is of the opinion that »it seems most probable that the fertility groups at least to some extent indicate a grouping after relationship and that the genetic differentiation is greater between groups than within, which again agrees very well with the fact that some of the partially sterile F_1's show an especially high degree of heterosis». The grouping of the lines in intra-fertile but partially intersterile groups does not coincide with a grouping of the same material according to the geographical or ecological distribution. Neither in *Galeopsis* nor in barley is there any relation between the degree of hybrid vigour in F_1 and the degree of geographical or ecological differentiation between the parents. This fact has been discussed in detail and is supposed to depend on the biology of distribution of the species in question (HAGBERG, 1952 b, 1953 b).

Several cases of structural heterozygosity combined with heterosis are reported in the literature. The classical example among plants is *Oenothera*, where a number of translocations has admitted the building up of stable blocks of genes, which are not broken up by crossing over. The blocks of genes are selected for heterozygosity and the highest possible degree of heterosis in these permanently and more or less partially heterozygous species. Similar cases are described in *Paeonia* (WALTERS, 1952) and structural heterozygosity is also found in *Godetia*, *Campanula* and other genera (*cf.* STEBBINS, 1950).

The most well-known cases in the animal kingdom of heterosis in connection with structural heterozygosity are those described by DOBZHANSKY (1948, 1949, 1950) in *Drosophila pseudoobscura* and by WHITE (1945, 1949, 1951) in *Trimerotropis*. DOBZHANSKY has shown that within one and the same population individuals, heterozygous for some of the studied inversions in the third chromosome, are selectively superior to individuals homozygous for the standard type or for the inversion in question. The inversion precludes crossing over and thus permits the building up of blocks of genes which react positively with other similar blocks. Such a system should result in heterosis only if gene blocks from the same population are combined and DOBZHANSKY (1950) has shown that, as a rule, no heterosis occurs in a combination of the corresponding inversions from two different populations, *i. e.* when the gene blocks have been selected in different populations. He writes: »Natural selection may have produced different polygene complexes in different populations, coadapted within but not between the populations». This is undoubtedly correct and the hypothesis may probably be extended to cover the development of dominance conditions in single loci (*cf.* FISHER, 1930).

It is necessary, however, to keep in mind the chronology of the different selective processes. It is possible to distinguish between a primary and a secondary selection, although the border-line between the two is by far not absolutely distinct. The selection resulting in heterosis because of the combination of polygene complexes, as described by DOBZHANSKY (*e. g.*, 1950), takes place after the occurrence of a structural change, a »chromosome mutation». This mutation has perhaps occurred more or less recently in the population and the selection within the polygene complexes may still be an actual phase in the phylogeny. This selection would be of a secondary type and presumes a primary process. This latter would mean a selection of genes in the species population and the evolution of the interactions and dominance relations. This is certainly an older and more primary process from a phylogenetic point of view.

The development of stable gene blocks giving heterosis and made possible by structural differences is only one special case, although extremely well suited to illustrate in what way the selection processes function in a population. Polygene complexes are built up, forming a unit and reacting as a block of alleles which in combination with another block gives superdominance or heterosis in the heterozygote. The building stones, the genes, and their individual effects and interactions

are older than the blocks. The new thing is the way in which they are combined because of the secondary process.

Evidently, a selection process of the type occurring in *Drosophila* cannot explain the relation between heterosis and disturbed fertility observed in *Galeopsis*. As mentioned above, the genetical differentiation *between* fertility groups is greater than *within* them, and the phenomenon is probably to be explained on the same basis as the occurrence of heterosis in crosses between genetically differentiated populations.

IV. THE MANIFESTATION OF HETEROSIS.

This section deals with the original questions (6) and (7).

Allogamous organisms are hardly suited for a detailed study of the heterosis phenomenon. For such a study of F_1, therefore, the author has used crosses within and between the two species *Galeopsis Tetrahit* and *bifida* (HAGBERG, 1952 a, b). In these autogamous species it is possible to work with »pure» lines and most of the lines here used have been grown by MÜNTZING for more than 25 generations under strict isolation and control. All individuals in an F_1 are genotypically identical, they form a biotype, and the variations within such a biotype are caused only by the environment. The material is, thus, well suited for a comparison between F_1 and its parents.

In order to obtain a fully comparable parent material artificial crosses were made between plants within the pure lines. No differences were found between pure selfing and crossing within the line. The intra-line crosses made it possible to compare the seed setting after intra-line pollination with that after inter-line pollination. The relation was most thoroughly studied in the autogamous, allotetraploid *G. Tetrahit*, and there was a clear indication that the seed setting was best in the inter-line crosses. Even in this autogamous species crosses are superior to pollination with isogenic pollen and this is supposed to be due to a superior vitality of the hybrid embryos which enable them to stand the adverse conditions incidental to artificial pollination better than the homozygous ones. In this connection reference is made to the discussion about autogamy in subsections III A and B, to BREWBAKER's (1952) studies on incompatibility in red clover, and to OLSSON's (unpubl.) on *Brassica napus* and tetraploid *B. rapa* and *B. oleracea*.

ASHBY (1930, 1936, 1937) pointed out that heterosis is to a large extent dependent upon the fact that the hybrid has an advantage already as a seed and a seedling. This observation is important, but there

is no marked correlation between the superiority in seed weight of F_1 over the parents and the final degree of heterosis in F_1. ASHBY has exaggerated the importance of the initial superiority. SPRAGUE (1936), on the other hand, is probably quite correct when he stresses that, since the size of the cells is not increased in a hybrid showing heterosis in a somatic character such as plant height, heterosis must be caused by an increased rapidity of the cell divisions in the meristems, in the vegetation points.

The increased rapidity of cell divisions is caused by an increase per unit time in the physiological activity, especially in the young seedlings (*cf.* RABIDEAU *et al.*, 1950).

Heterosis is, however, not only the result of an increase in the rapidity of cell division. The growth rhythm during different stages of the development varies often very much between different biotypes. The differentiation of initial cells and the formation of new vegetation points, *e. g.*, branches, also vary from biotype to biotype. The fairly constant growth scheme, typical of a certain line, is inherited and F_1 combines the genetic factors from both parents. Usually the type most favourable to general vigour and competitive ability in F_1 is dominant, and in many cases the factors, as well as the characteristics of the growth schemes determined by them, combine in a way which is favourable to the general vigour of F_1. In many cases it can be directly demonstrated that the hybrid vigour is due to a combination of characters from both parents — combination heterosis according to HAGBERG (1952 b). There is, however, also a type of generally increased growth rate which cannot be analysed into part characters and which HAGBERG (*l. c.*) has called transgression heterosis. (This term applies to F_1 and is not to be confused with transgressive segregation in F_2, although the two phenomena are causally closely related.) Usually transgression heterosis is apparent in the growth rate but it may appear also in regard to other characters. JONES (1921, 1952) seems inclined to limit the term heterosis to transgression heterosis in the growth rate.

In his papers on *Galeopsis* the present author (*l. c.*) also discusses a type of »heterosis» which is a result of a supernormally long continuation of vegetative growth, due to a more or less marked sterility in the heterozygote. This is, however, definitely another thing than the directly caused somatic heterosis and perhaps it should not be counted at all as heterosis. Possibly it is this type of luxuriance which has caused the reluctance of DOBZHANSKY and others to accept any type of luxuriance or somatic heterosis as true heterosis.

Combination as well as transgression heterosis has been illustrated by several examples especially from *Galeopsis*. In cases of combination heterosis it is possible to analyse at least to some extent the mechanism which results in hybrid vigour. The mechanism of transgression heterosis is considerably more difficult to analyse. Even in this case it is, however, most probably a question of a combination of genetic factors, only these factors and their effects are at present unavailable for the analysis. It would obviously be desirable to analyse the more or less complex characters studied into their ultimate elements — the genetic factors. JONES's theory of 1917, which on the whole is still the most generally accepted theory of the cause of heterosis, assumes this to be caused by a combination in F_1 of a large number of linked, dominant genes, conditioning an increased vigour. Anyway, this mechanism certainly is one of the major causes of heterosis. In combination heterosis the combining factors can be partly analysed, divided into groups influencing part characters; in transgression heterosis there is no such possibility and the phenomenon is apparently more complex. There is, however, no defined limit between the two types and theoretically they are but different expressions of the effect of the same mechanism.

So far the present discussion has accepted the theory of dominance. As early as in 1918 FISHER pointed to the possibility of superdominance in a combination of two alleles belonging to the same locus. Such a superdominance is in itself hardly essentially different from the phenomenon of complementary factors. In both cases it is a question of an interaction between genes, in the one case alleles in the same locus, in the other non-allelic in different loci. And by duplications the two alleles of one locus may be located in non-homologous parts of chromosomes and thus lose their allelic relations and change into different genes with similar effect. The same may be the case in polyploids, as discussed above. If the possibility of a position effect is disregarded the biochemical and physiological reactions and interactions will be the same whether the two genes are alleles or belong to different loci. The occurrence of superdominance would make it still more difficult to analyse cases of transgression heterosis, but fundamentally it would even then be a case of combination heterosis, as will be further discussed in section V.

In his papers on *Galeopsis* the present author (*l. c.*) has discussed the variability of F_1 in comparison with the parent lines. Three essentially different types of variability may be distinguished:

(1) *The variability within the plants, e. g. in cell size.* — In one of the author's cases F_1 was more variable than the parents. This may be taken as an indication that in some instances F_1 may be more unstable, may have lost some of the finely adjusted internal balance of the parents.

(2) *The variability between plants within the biotype.* — In the author's *Galeopsis* material there is no tendency for the F_1 plants to be either more or less variable, *i. e.* more or less influenced by or dependent upon the environment, than the parental biotypes. GUSTAFSSON (1946) found a greater »plasticity» in heterozygotes in barley than in the corresponding homozygotes, *i. e.* the heterozygotes reacted more strongly than the homozygotes upon changes in environment. On the other hand, it is theoretically conceivable that »plasticity» might mean a superior adaptability to different environments and if such were the case it must not necessarily result in a wider variability in F_1.

(3) *The variation between F_1 biotypes.* — In this case the variation in the whole series of F_1's is compared with the variation in the corresponding series of parents. The series of heterozygous biotypes shows a more narrow range of variation than the corresponding series of means of parental pairs.

It is also of a considerable interest to study the relations between the variation curves in different characters. As shown and discussed in detail by HAGBERG, 1952 b, p. 241, we meet two distinctly different types of dominance. In regard to height and dry matter the F_1 curve reaches at least as high values as the curve of parental means but not the low values of this curve; in regard to earliness neither the positive nor the negative end of the parental curve is reached by the F_1 curve. This has caused the author to draw the conclusion — perhaps too speculative — that great height and high dry matter production on the whole have had a positive selective value (compare the discussion on page 357 concerning the reason for the high competitive ability of the allopolyploids) while extreme earliness or lateness during the phylogeny of the species has, on an average, probably been selectively disadvantageous. The main part of the biotype, the balanced interactions of the genes and the dominance relations have been formed by selection over a long period of time and probably mostly in the centre of the area of the species, where extreme earliness or lateness would be selectively disadvantageous.

MALINOWSKI (1952) has studied the variation between individuals in F_2 of crosses which have shown heterosis in F_1, and he considers it

to be possible to estimate approximatively from the shape of the F_2 distribution curve the number of factors which have been combined in F_1 to bring about heterosis. Among others he has analysed the data from MÜNTZING's (1930) crosses between *Galeopsis Tetrahit* and *bifida*, and from the shape of the F_2 distribution curve he draws the conclusion that two complementary factors are responsible for the luxuriation of the species hybrid. Having worked with the material in question the present writer can only consider the conclusion as untenable.

In several cases in the material of the present author uniform biotypes have given distribution curves — purely modificative variation — which according to MALINOWSKI should have indicated segregation in two factors.

Against the general idea of MALINOWSKI it may also be stressed that the effect of the genes most probably is not directly added. Already twenty years ago RASMUSSON (1933) pointed out that the effect of each new gene added would probably decrease with an increasing number of genes with a similar action present, and recently FISHER (1949) has laid stress upon the fact that if the height or yield of a plant is near a »ceiling» the relative effect of each factor conditioning height or yield becomes less. The present author has studied the variation curves in F_2 in some *Galeopsis*- as well as barley-crosses but has been unable to draw any definite conclusions, nor have the curves added anything to the understanding of the phenomenon of heterosis.

In certain cases there are differences between two reciprocal crosses (*cf.* MICHAELIS, 1951). If the *Galeopsis Tetrahit* line T—B is used as mother in crosses with *G. bifida* the germination is very much delayed and many seeds do not germinate at all. With T—B as father the same combinations germinate rapidly and show a marked heterosis in the seedling stage. Probably a substance inhibiting germination is produced in excess by the combination of one *Tetrahit* and one *bifida* genome when working in the plasm of T—B. The delayed germination influences the further development of the plant and without a careful study of the germination conditions we would have had an excellent example of different degrees of heterosis in two reciprocal combinations. If, however, the seeds with T—B as mother are brought to rapid germination the heterosis is equally strong in both reciprocals.

Several cases of differences between two reciprocals in the degree of heterosis are reported in the literature and summarized by MICHAELIS (1951). This author points out the great importance of the cytoplasm as

carrier of inherited characters and quotes cases of »plasmonabhängige Heterosis». In experiments with *Drosophila* GOWEN (1946, 1952) has found that »diverse cytoplasm has shown less yield than like cytoplasm» and he is of the opinion that »hybrid vigor is attributable to nuclear contributions of the two parents rather than to possible cytoplasmic differences in the uniting gametes». HERIBERT NILSSON (1937) is of the opposite opinion and means that inbreeding degeneration in rye is due to homoplasmony and heterosis to heteroplasmony.

During the embryological development and until the ripening of the seed the young zygotes develop in direct dependence of the mother plant. In many cases the latter may give the progeny characters which are not further inherited but which are an after-effect of the genotype of the mother. (Compare, *e. g.*, the fact that a plant of yellow lupine which is genetically nearly free of alkaloids but the seed of which developed upon a bitter plant will remain bitter towards ripening but will produce »sweet» seed; HAGBERG, 1950.) The case of *Galeopsis* T—B may have a similar explanation, as perhaps also other cases of reciprocal differences. Probably the importance of the cytoplasm has been overrated as well as underrated. No doubt it plays a considerable rôle in the intricate interactions between genotype and environment but as its effect is often difficult to ascertain by experiments it has, unfortunately, often been overlooked.

V. DOMINANCE AND SUPERDOMINANCE.

In the attempts to explain the heterosis phenomenon it is usual to discuss individual genes, not the polygene complexes. There are two different opinions about the interactions of the two alleles of a pair: the dominance theory, which may be symbolized as $AA \geqq Aa > aa$, and the superdominance theory, which may be symbolized as $Aa > > AA > aa$. Our knowledge about the real nature of the genes and their mode of reaction is still rather scant. Studies on certain morphologically distinct and fully vital X-ray mutations — some of which even are superior to the mother line in reproductive capacity under normal conditions at Svalöf — may perhaps contribute something to our knowledge of the reactions of the genes. The author has studied the erectoides mutations in barley (HAGBERG, 1953 a).

The fact that breaks in the chromosomes and small or large deficiencies are common in X-ray mutations introduces a possible source of error in studies on this kind of material. As long as mutation has not

been shown to be caused by a deficiency or to be solely dependent upon some other type of structural change and if genetically it behaves as a normal genetic factor, the author has considered its permissible to treat it as a factor mutation (more or less synonymous with a point mutation).

In this connection the author wants to point out that in these discussions he is using the term *gene* in the sense which DAWSON and WHITEHOUSE (1952) consider to be the only correct one: the term gene indicates *one* hereditary unit and is not — as used by several authors — a collective term including a series of alleles and synonymous to locus. The different members in a series of multiple alleles are different genes, located in the same locus. Reference is made to the discussion on page 364 and to the following discussion.

In the homozygous state the factor mutations studied very often have a pleiotropic effect on the phenotype (*cf.* CASPARI, 1952). As a result it is sometimes difficult or impossible to classify these mutations as strictly dominant or recessive. In regard to one character the mutant factor may be recessive, in regard to another it may be dominant, and in the case of a third character the normal allele may show superdominance (see further HAGBERG, 1953 a). One factor mutation may at the same time have both positive and negative part effects upon the phenotype. Sometimes two alleles seem to function as complements to each other, thus causing monofactorial heterosis.

Superdominance may be caused by two different types of interaction between the alleles: (1) the two alleles function as complements, as just described; together they result in a better effect in the heterozygote than each of them in a homozygous state are able to produce. (2) One of the alleles may be without effect, may even be a deficiency — the other allele has an optimal effect when in single dose and the homozygous state is an over-dose (*cf.* FISHER, 1918; HULL, 1952). It is possible, on the other hand, that lethal or semilethal recessive genes may have a similar mode of reaction themselves; a single dose giving an optimal development. In an allogamous population the selection must often result just in an optimum for the single dose. To this may be added the effect of the other allele, since it seems probable that both types of reactions and interactions may occur simultaneously.

The two types of effects and interactions and the fact that a gene has often a pleiotropic effect upon the phenotype makes it very probable that certain loci may show superdominance, especially in more complex characters. (Compare the discussion of transgression and com-

bination heterosis on page 364). The word »complex» is used to denote characters which are the result of a more or less complicated combination of more simple, primary ones; the latter may then again be genetically fairly complex. Such characters as yield of dry matter, *i. e.* total somatic production, reproductive capacity and adaptivity are extremely complex; height, number of florets, cold resistance, *etc.* are one degree less complex (*cf.* HAGBERG, 1953 b).

It seems to be a rather prevalent opinion that the theories of dominance and superdominance are more or less reciprocally exclusive. The author cannot consider such an opinion to be correct. During evolution the amount of chromosome substance has most probably increased not only by polyploidy but also by duplication, as discussed before. Genes which during one stage of the phylogeny have been alleles may become multiple genes or complementary genes. Physiologically and biochemically the genes should interact — disregarding the possible position effects — in the same manner whether they are alleles or multiple or complementary genes in different loci. Complementary genes give together an effect which each of them alone cannot produce. It seems reasonable to assume that two alleles may be complementary in a similar manner, and there is nothing astounding or »unnatural» in the phenomenon of superdominance. Rather, it would be »unnatural» to assume that it could never occur.

As pointed out by the author (HAGBERG, 1953 a), it is at present impossible to prove unequivocally by experiments the occurrence of superdominance, since we do not know the nature of the gene, nor its biochemical functions. MÜNTZING's (1945 b) criticism of the case of superdominance in *Antirrhinum majus* reported by STUBBE and PIRSCHLE (1940) may partly be directed against any case of superdominance in spontaneous or artificial mutations. Besides the case mentioned there are several other instances of one-factor-heterosis described in the literature: JONES, 1921, 1945; ROBERTSON *et al.* 1932, 1935; GUSTAFSSON and coworkers, 1946, 1947, 1950, 1952; STERN *et al.* 1952; QUINBY and KARPER, 1946, 1948; BUZZATI-TRAVERSO, 1952; TEISSIER, 1942, *etc.*; STADLER, 1939; NYBOM, 1950; HAGBERG, 1953 a, and many others. There are also, however, monohybrid combinations in which no superdominance was found, *e. g.* in some mutations in lupine (TEDIN and HAGBERG, 1952) and in several barley mutations (HAGBERG, *l. c.*).

In his studies on *Neurospora* heterocaryons EMERSON (1948, 1952) has found that a phenomenon very similar to one-gene-heterosis sometimes occurs. His cases may probably be interpreted as caused by the

second type of mechanism for superdominance discussed above. From his whole experience of the *Neurospora* material, however, EMERSON sums up: »There is nothing in the studies of heterocaryosis in *Neurospora* to suggest that one-gene heterosis is of general occurrence and importance».

The blood group alleles which produce blood group antigens in man independently of the other allele in the pair are, together with several other phenomena in serology, a very good example of the situation that each allele directs the production of a certain substance and that the products are added.

The relations between the blood group alleles throw a certain light on the concept of dominance, since in this case it is impossible to speak of dominance or recessivity at all. The conditions are somewhat similar in regard to the erectoides mutations, in the case of which their pleiotropic effect made it more or less meaningless to speak about dominance or recessivity of a given factor mutation. It may be said, however, that the mutation or the normal allele is dominant in its effect upon some »primary», uncomplicated character. Dominance is to be expected when the effects of the two alleles are antagonistic or when the effect of one of them is 'epistatic' over the effect of the other. Primarily, the cause of dominance for one allele must depend upon chemical or biochemical relations. Often natural selection leads the evolution of dominance relations in a direction favourable for adaptation (*cf.* FISHER, 1930). The processes of selection take place in allogamous populations and the whole genotype with its actions and interactions is adapted to heterozygosity. It is then easy to imagine that two alleles may complement one another and the sum of their effects upon the development of the heterozygote will in some cases show superdominance, in other complete or partial dominance. Partial dominance is perhaps the most common relation between two alleles. The symbols used at the beginning of this section to denote dominance or superdominance should, in fact, be replaced by the general symbolization $A_1A_1 \lessgtr A_1A_2 \lessgtr A_2A_2$.

VI. CONCLUDING REMARKS.

An attempt at a final summarizing discussion would mean an unnecessary repetition since this paper is a general discussion of the empirical results and conclusions published by the author in previous papers. Instead, an attempt is made at some final commentaries on the discussion about some of the aspects of the heterosis phenomenon.

When DOBZHANSKY (1950) points out that there are two kinds of heterosis or euheterosis (his »luxuriance» is then not included in the heterosis concept) his statement is founded upon the present situation in *Drosophila pseudoobscura*. »The first kind arises from the presence in populations of deleterious recessive mutant genes sheltered by their normal dominant alleles. Accumulation of these deleterious genes is a by-product of the mutation process.» He calls this type of heterosis mutational heterosis and it is the most important one if the heterosis phenomenon is turned around and inbreeding degeneration is studied. »The second kind of heterosis is due to complexes of linked polygenes which give specific 'heterotic' interaction effects in heterozygotes.» »This kind of heterosis is engendered by natural selection as a form of adaptation of the species to its environment.» DOBZHANSKY calls this type balanced euheterosis. He states (1952) that »balanced heterosis is a result of overdominance».

Out from DOBZHANSKY's results with *Dr. pseudoobscura* and his two types of heterosis, mutational and balanced, and from the fact that somatic heterosis, appearing as hybrid luxuriance, has been shown to be at the same time adaptive heterosis, *e. g.* in amphidiploids, the author has tried to differentiate two phases in the process of natural selection, one primary and one secondary. During the primary process the selection directly concerns the material of genes made available by the mutation process as gene mutations—point mutations. It includes a selection of individual genes whose effects and interactions result in the best possible development of the individual, high vigour and competitive ability. Genes with an unfavourable effect are either rejected or they become recessive.

A secondary selection occurs as the result of a chromosome mutation, *e. g.*, an inversion as in the *Drosophila* material. Its working material are the genes selected in the primary process and its result are the balanced gene complexes. The author is well aware that the distinction between primary and secondary selection is rather grossly schematic, but he considers it, nevertheless, to be of value for an understanding of the heterosis phenomenon.

Selection does not occur only at present but has been active as long as populations of living organisms have existed. Many genes have been selected very early during phylogeny, before the original mother population was split up into what we now consider to be separate species. The majority of the genes have been selected during a very long series of generations and the effects and interactions have reached a fairly

balanced state in most of the »good species» of today. The secondary selection resulting in balanced blocks of genes has to work mainly with these old genes and their reactions and interactions which are already to a very large extent adapted to the genotype as a whole. As mentioned earlier in this paper, the assumed cause of these balanced blocks is often the appearance of certain types of chromosome mutations, which prevent viable recombinations in the heterozygote between genes in the changed segment. The primary and secondary selection processes are not, as already pointed out, distinctly separated but more or less interwoven; indeed, a selection of the primary type will go on even after a structural change has made the secondary selection possible. Secondary selection after chromosome mutation has so far been shown to occur only in a few cases. On the other hand, many of our »»single factors» may as a matter of fact be more or less complex »gene blocks».

According to the deductions here made the primary selection, and especially its early phases, are the fundamental cause of hybrid vigour in species hybrids. The somatic heterosis occurring in such hybrids is, perhaps, the most typical instance of »luxuriance». Obviously, it has no direct relation to adaptation and in cases of complete sterility it is without any importance for the evolution. If the chromosome number of a species hybrid is doubled, however, the result is an amphidiploid which in many cases — if the reproductive capacity is sufficiently established — may, besides or because of the luxuriance, have a high degree of competitive ability and the appearance of which marks an important step in the evolution. The luxuriance of the species hybrid is brought about by complementary interactions of genes from the parent forms. The interaction of these genes, again, depends upon the fact that they have been selected *together* (in the same population) during some earlier phase of phylogeny.

The high competitive ability of many amphidiploids confirms the opinion that hybrid luxuriance is not unrelated to euheterosis; that it is not a question of two definitely different phenomena. From the breeder's point of view the somatic heterosis is of the greatest importance and with very good reason most breeders decline to consider it as a specific phenomenon, separated from the real heterosis complex.

CROW (1952) expresses about the same opinion as DOBZHANSKY: »It is questionable whether the increase in size that is sometimes observed in variety hybrids is really adaptive». Obviously it can not always be. Sometimes the »ceiling» will be reached. Sometimes the

hybrid is sterile and the sterility prevents further adaptive selection. The main point is that the genes which cause heterosis have been selected as to effects and interactions in a state of heterozygosity and that, therefore, they are »dominant». The two different types of dominance relations shown in the *Galeopsis* material for such characters as flowering and ripening time on the one hand, height and total production on the other hand offer a further argument in the discussion and an argument against the idea that luxuriance or somatic heterosis is essentially different from »euheterosis».

In section V dominance and superdominance have been discussed in detail together with the different interactions which may result in superdominance and their evolution by means of selection. By the aid of mathematical computations and by postulating certain given conditions, which may not always be realized. CROW and HULL and others have reached at the conclusion that superdominance is necessary to explain the degree of heterosis found. Even a very limited number of loci with superdominance would be sufficient to explain this heterosis. HULL (1952) discusses the possibility of a pseudo-overdominance with effects identical to those of true superdominance. In his studies on hybrids in barley and tomato POWERS (1952) has found no case of superdominance; he interprets his results as cases of combination heterosis. Nor has MATHER (1949) found superdominance in barley.

The opinions vary widely about the importance of the rôle played by superdominance in the occurrence of heterosis. But the diversity of opinion goes still deeper. With different points of view from the beginning and with different detail problems to solve, different authors reach at widely different opinions even in regard to the concept and definition of heterosis as such. It has been impossible to get an unequivocal definition accepted in spite of all the attempts made to elucidate the problem. The reason for the lack of success in this respect is probably the fact that the whole phenomenon is too complex for us to grasp with our present rather schematical methods of analysis and sets of symbols. Our knowledge of the nature of the genes, of their reactions and interactions with the biochemical and physiological environment is very uncomplete, not to say non-existent. We cannot prove conclusively the occurrence of superdominance, nor are we always able to distinguish with certainty between pseudo-alleles and true ones.

It is improbable that the biochemical and physiological mechanisms

involved in the gene reactions are of a uniform type. Rather, there must be numerous different mechanisms by which the genes form their gene products and ways in which they react with other genes, their gene products and changes in the environment.

All cases of heterosis have one thing in common: the motive force, *viz.* the selection of biotypes with the highest possible degree of adaptation (*cf.* DOBZHANSKY, 1949). By selection the different modes of reaction are tried. The end result of the selection is the uniform or fairly uniform behaviour of the end product, the more or less heterozygous hybrid with hybrid vigour. The uniformity in behaviour is superficial, and the »conformity to laws» perhaps only apparant. The means to attain the end product are probably very different in different cases, in different organisms and in different types of genes in one and the same organism.

In all cases of heterosis two genotypically different gametes are brought together. Different genes, which may complement each other in different ways co-react in the formation of the individual in question. The co-reaction is perhaps mainly of the type presumed by JONES (1917) in his theory, but more complicated modes of interaction, among them superdominance, are not excluded. The essential thing is that the heterozygote is in possession of a more rich material of genes, there are more individual elements and, thus, a greater possibility for variations and combinations.

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SUMMARY.

(1) This paper gives a summarizing discussion of the results presented in previous papers by the author.

(2) The concept and definition of heterosis are discussed. The author maintains the definition of heterosis given by SHULL; it is measured by the difference between F_1 and the superior parent.

(3) Heterosis is found among self-fertilizers as well as in cross-fertilizers. It may occur in crosses between populations of the latter. The occurrence of allogamy in early phylogenetic stages of autogamous species is supposed to explain the occurrence of heterosis in autogamous species.

(4) Heterosis is found in wild and in cultivated species. In cultivated species it does not occur in characters for which strict positive selection has been made by plant breeders but is apparent in other characters where no such selection has taken place. In both groups of characters the inbreeding effect, found by comparing F_1 with the following generations, is essentially the same.

(5) Crosses within and between the allotetraploid species of *Galeopsis* give a more marked heterosis than crosses within and between the diploid species. Autotetraploids of barley, on the other hand, show the same degree of heterosis as the corresponding diploids.

(6) In allotetraploids the gene interaction which results in heterosis in species hybrids is fixed, which is supposed to explain the superior competitive ability often shown by allotetraploids. In this case the »luxuriance» is a somatic heterosis which at the same time is adaptive.

(7) Heterosis occurring in connection with structural heterozygosity is discussed; the cases found in *Galeopsis* are fundamentally different from those in *Drosophila.*

(8) Many cases of heterosis depend upon an increase in the rate of cell division. The author has distinguished between combination and transgression heterosis, both of which, however, are the result of recombination of genes.

(9) On comparing the variability of F_1 with that of the parents there are three fundamentally different types of variation to be considered: within plants, between isogenic plants and between the biotypes in populations of F_1 or parents. In respect to the last type the relation between F_1 and parents is different in different characters, which fact is taken to indicate different dominance relations. These, again, are supposed to be the result of differences in selective value.

(10) The author maintains that dominance and superdominance may both exist simultaneously and contribute to heterosis. The relation between the two phenomena is discussed in detail.

(11) The author distinguishes two phases in the process of selection. The primary one deals with single genes — point mutations — the secondary one with blocks of genes which are made permanent by chromosome mutations.

(12) The heterosis phenomenon is probably not dependent upon one single cause or set of conditions but is the result of extremely complicated interactions. It depends, however, upon the selection of biotypes with superior competitive ability. F_1 has a larger number of genes which may interact with and complement each other than have the parent lines.

Svalöf in December 1952.

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[An exhaustive bibliography on the subject heterosis is to be found in the Proceedings of the Heterosis Conference at Ames, Iowa, edited by J. W. GOWEN (1952)].

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CYTOGENETIC INTRASPECIFIC STUDIES IN LAMIUM. II

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THE present paper is a direct continuation of, and was prepared at the same time as, a first part which has already been published (BERNSTRÖM, 1952). As to the purpose of the investigation, the methods employed, *etc.*, reference is made to the section previously published.

IV. EXPERIMENTAL.
(Continued.)

2. L. PURPUREUM.

Fifteen pure lines of this species were used in crossing experiments, and several more were cultivated. The 15 lines are:

*p*1 From the neighbourhood of Gothenburg, Sweden.

*p*2 Ditto. Both these lines are tall, with the most convex leaves within the collection. The uppermost leaves in *p*2 are, however, richer in anthocyan than in *p*1, and the flowers are also darker than those of the latter.

*p*25 From Lund, Sweden. Fairly like the two above-mentioned lines but low-growing, and the shape of the flowers and the markings of the lip are different.

*p*26 From Waadt, Switzerland. Stiffly upright, light green. The pollen lemon-gold, in the other lines orange-red.

*p*29 From the neighbourhood of Malmö, Sweden. Is recognized by the size and density of the flower pattern, its rather low height, and its light green colour.

*p*31 From Central Bohuslän, Sweden. The flowers are pure white. Much later to begin flowering than the other lines, especially in the autumn.

*p*51 From Lund. The flowers are white with violet spottings on the lip, and violet streaks on the tube.

*p*53 From Lund. The flowers are rosy instead of the usual purplish-red colour of the species.

*p*54 From Lund. The flowers are white, with 1—2 pale patchy markings on the lip, which is very often double, *i. e.* 4- instead of 2-lobed.

*p*71 From Sverdlovsk, Russia. Resembles *p*2, but with a different flower pattern.

*p*85 From Zahleh, Syria. Flowers 1—1 1/2 month later than the majority of the lines. Tall, richly branched, multi-internodical, of a type which differs from that of the other lines, although clearly belonging to the species.

*p*96 From Koralpe, Austria. The most low-growing line of them all.

*p*97 From Antwerp, Belgium. Distinguished by some minor characteristics, *i. a.* an unusually rich indentation of the leaves.

*p*98 From Rouen, France. Of ordinary appearance, without any clear distinctive features.

*p*99 From Graz, Austria. Together with *p*85 the tallest of the lines.

Several other lines than those dealt with above were cultivated and found to include, *i. a.*, those types with different periodicity which MÜNTZING (1932 b) was able to prove experimentally. Thus, some of the lines proved to be typically summer-annual, like most of those used in the experiments. Others were either more or less winter-annual or short-day-flowering because when cultivated in summer they refused for a long time, or even entirely, to flower, and they generally developed only a profusion of multi-internodical branches. Thus, certain lines from Southern Europe and South-Western Asia may flower, to a limited degree, in the summer 2—3 months after sowing; *p*85 belongs to the more quickly developing and richly flowering among these. *L. purpureum albiflorum II*, described by MÜNTZING, although he did not know the origin of it, should obviously also be classed in this group. Other lines from the same areas entirely refuse to flower during the summer in which they are sown.

Of a total of 28 samples of *L. purpureum* from abroad which were cultivated tentatively, 14 were found to be summer-annual, thus flowering 1—1 1/2 month after sowing, while the other 14 refused to flower or, generally, flowered unsatisfactorily and late after an abundant vegetative development. The samples from the former group originated from Austria, Switzerland, Holland, Belgium, France, and Northern Russia, while those of the latter group were obtained from Portugal, France, Switzerland, Hungary, Italy, Roumania, Southern Russia, and Syria. The two types evidently represent different geographical areas,

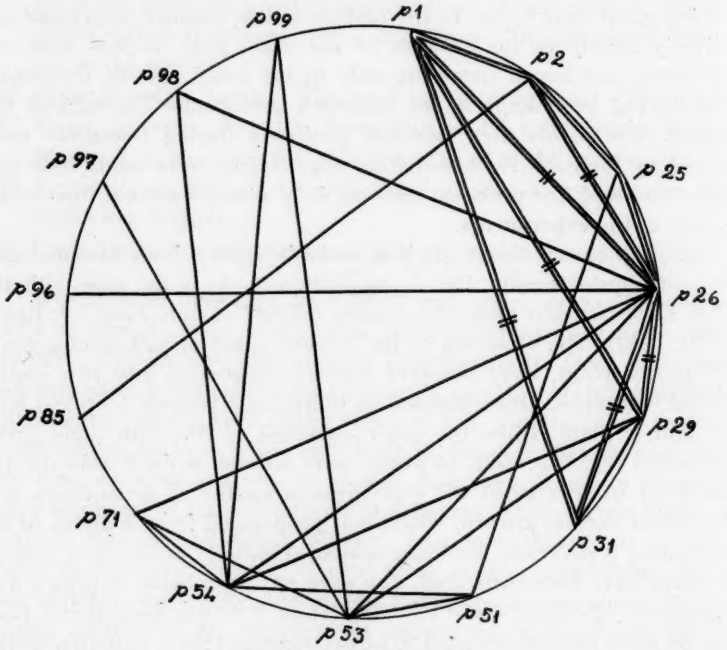
and meet at least in Switzerland and France. There are also types which may be regarded as intermediary between the two groups, as far as the rate of growth is concerned. Thus, *p*31 is almost as early to flower as the summer-annual lines when cultivated in the early summer while, in the autumn, it is 1—2 weeks later than the others. Another entirely white-flowered line, from which seeds were gathered from a wild-growing plant near Lund, in the middle of the summer, developed exceedingly slowly in the autumn of the same year. It was sown on July 22nd, and began flowering only in the early part of December, after having been kept in an unheated greenhouse throughout the autumn. The plants then flowered profusely during December until stopped by the cold. No hibernation experiments were made with any of the lines and, for practical reasons, only summer-annual lines could be used in the experiments.

L. purpureum, therefore, is a variable species both morphologically and physiologically, like *L. amplexicaule*. As is the case with the latter, *L. purpureum* probably consists entirely of pure lines (*cf.* BERNSTRÖM, 1952). The offspring of the original plant of *p*29, which was a well grown plant, were however found to segregate into two clearly defined categories. One consisted of individuals entirely identical with the mother plant, while the other consisted of dwarfish, light green plants (see Fig. 1, p. 393). 18 plants were normal, while 3 were dwarfs. The latter have retained the type through another 5 generations, and also *p*29 of normal growth, which was propagated from 3 plants of the segregating generation, has since remained entirely constant; a total of 80 plants have been cultivated. The ratio 18 : 3 probably implies a 3 : 1 segregation ($\chi^2 = 1{,}285$; $0{,}3 > P > 0{,}2$). Thus, it would seem that a plant was, by mere chance, revealed to be heterozygous for a mutation which, in all probability, had occurred in some earlier generation.

The mutants which have been labelled *p*29b, flower 3—16 days later than *p*29, differing in various generations, and wither 2—3 weeks later than their original line. During an early stage of growth, they have the same green colour as *p*29, but, while this remains constant, does that of the mutants get lighter until, at the time of flowering, they clearly differ from *p*29. The poorer plant development is probably caused by this chlorophyll deficiency; the seed-setting, too, is more or less inferior to that of *p*29, while the pollen fertility is perfectly good. The mutants develop and set seed much better in shade than in sunlight.

A. FERTILITY.

As in *L. amplexicaule*, interlinear F_1 hybrids were produced with a view to tracing possible chromosome-structural or unexpected genic or plasmatic differences between the lines (*cf.* BERNSTRÖM, 1952). Possible differences between the lines, particularly such of a chromo-



Scheme 1. (See the text.)

some-structural kind, should be known when judging the various species hybrids in which they have been used.

Interlinear hybrids given in Scheme 1 were studied. Single lines indicate hybrids produced by one-way crossing; double lines indicate that reciprocal hybrids were studied during at least one generation. Two cross-bars across the line or lines indicate that the hybrids were studied during two generations.

No clear differences were found between reciprocal hybrids, at any time or in any respect, and their values were therefore pooled.

L. purpureum corresponds to *L. amplexicaule* in the very high and

even pollen fertility of the pure lines, irrespective of the season. Only in exceptional cases is the percentage of empty pollen grains > 5 in a single plant. The pollen fertility was studied in 10 of the pure lines used for intraspecific crossing experiments, during 1—7 (*p*26) generations, and the total number of plants investigated in each line varies between 5 and 71. The separate mean values of the percentage of good grains vary between 96,6 (*p*26) and 99,8. The average of all the mean values for pure lines within *L. purpureum* is $98{,}6 \pm 0{,}118$; n=39.

In sharp contrast to the conditions in *L. amplexicaule*, hardly any reduction of the fertility was found in the hybrids within *L. purpureum*. This has previously been reported (BERNSTRÖM, 1950 a), but there only those hybrids are mentioned which were examined also with regard to the seed-setting. The pollen fertility of the hybrids expressed as a percentage of the mean value of the averages of the parent lines varied, in all cases where all the three parties were simultaneously investigated, between 98 and 102; the distribution is $3 > 100$, $12 = 100$ and $6 < 100$, these values comprising 16 different hybrid combinations. To these should be added 11 other combinations concerning which both parent lines could not be investigated during the same generation; in these hybrids the mean values vary between 94,5 and 99,3.

The former figure, which is remarkably low, is based on only two plants of *p*31×*p*1 from 1945—1; in a previous generation, the same hybrid combination had a mean value of the pollen fertility of 98,7, based on 13 individuals. Therefore, it is hardly probable that the lower one of the mean values is characteristic of these hybrids; and apart from this one, the lowest mean value found in any hybrid combination is 95,8 (n=13). This refers to *p*2×*p*26—44—2, and constitutes 98 % of the average of the mean values of the parents. The same combination, in another generation, had a pollen fertility of 98,3 (n=14), corresponding to a relative value, as above, of 101. Nor is there, in the case of the mean value of 95,8, consequently any reason to believe that it implies a real, genotypically conditioned reduction of fertility. The second lowest value of pollen fertility in any hybrid generation is 97,8.

The seed-setting of *L. purpureum*, so far investigated, has been described elsewhere (BERNSTRÖM, 1950 a). It was found to be so good in the hybrids as to be, most probably, even superior, on an average, to that of the pure lines. The figures relating to the seed-setting are, however, somewhat lower throughout than those representing the pollen fertility (*cf.* BERNSTRÖM, 1950 a, Table 1); still, this could be expected as a consequence of the pollination having occasionally failed, or

having been too scarce, or of other disturbances in the process leading to fertilization. Evidently, the seed-setting varies to some extent between the lines, *p*26 from Switzerland having apparently a seed-setting inferior to that of the other lines investigated. This may possibly be a result of adaptation to a climate different from ours.

The full fertility of the intraspecific hybrids shows that chromosome-structural or possibly other differences between the lines, which are of a nature to produce sterility, and which are common in *L. amplexicaule*, do not occur in the material of *L. purpureum* investigated. Since this was collected from so many different regions, and since it also comprised different ecotypes — by which is meant *p*85 in contrast to the others — it seems permissible to presume that chromosome-structural differences between the lines are, on the whole, rare in this species. The discovery of a plant with a ring-shaped chromosome in a pure line of *L. purpureum* (GRIESINGER, 1937) indicates, however, that chromosomal aberrations may appear spontaneously.

B. CYTOLOGY.

The number of chromosomes was counted in root-tips and was found to be $2n=18$ in 31 different lines of *L. purpureum*, among which were all those used in the crossing experiments.

The reductional division in diploid *L. purpureum* has not been examined in detail. Fixations in NAVASHIN's solution gave bad results; they did only show that meiosis appears to proceed quite normally, but did not render closer studies possible.

C. MORPHOLOGY.

In the corresponding chapter relating to diploid *L. amplexicaule* (*cf.* BERNSTRÖM, 1952) an account has been given of the purpose of the measurements which were made on this species, as well as of the procedure. *L. purpureum* has been examined in approximately the same manner; measurements corresponding to those on *L. amplexicaule* were made on *L. purpureum*, except in the case of the leaves; here the length of the lamina itself was measured, the petiole was not included, being well developed in this species. In *L. purpureum*, the length was also taken of the lowest pair of branches, *i. e.* that extending from the axils of the cotyledones. Further, the numbers of teeth were counted on the leaves measured; the number of teeth is greater than in *L. amplexicaule*, and was presumed to reflect any differences in the size of the respective leaves.

All generations of *L. purpureum* measured were kept in open frames, covered with glass panes. As to the results, no clear difference was found in any characteristic between reciprocal crossings; their values have therefore been added together. The plants of 1945—2 were, as in *L. amplexicaule*, too withered to allow any measuring, when the opportunity arose. Only height measurements could be made; the commencement of flowering was also studied. There was no time for measurements of the 1946 material at all.

The values obtained are reviewed in Table 1. The means of the hybrids are also expressed in percentage of the mean of the average values of the parent lines. Height of stem, and length of branch, are also computed in percentage of the tallest parent line. Mean values exceeding that of the greatest parent line, or the earliest with regard to the commencement of flowering, are in italics. The methods used in estimating the time of flowering were the same as in the case of *L. amplexicaule* (*cf.* BERNSTRÖM, 1952, p. 212).

Table 1 shows that hybrids within *L. purpureum* are characterized by a general tendency, similar to that of *L. amplexicaule*, towards superiority over the mean value of the averages of the parents; it also shows that this tendency is often strong enough to cause the measurements of the hybrids to exceed those of even the largest parent line — in other words, real heterosis does exist, in the sense of SHULL (1948). This applies, with the fewest exceptions, to the height of the plants; no hybrid presents a lower relative height value than 104, and even in 1944—1, which was characterized by the smallest relative growing capacity of the hybrids, this was just as marked as during the other generations. Irrespective of such a possible influence on the extent of the capacity of growth of the hybrids, which may have been effected by unequally extensive hybridization with the different lines, the probability is about 1 : 130000, *i. e.* $\left(\frac{1}{0{,}5^{17}}\right)$ that the height of the hybrids in percentage of the arithmetic mean of the height of the parent lines should be > 100 in all the combinations, instead of an even distribution around 100. Thus, the tendency towards heterosis is highly significant while, on the other hand, a common existence of real heterosis is more uncertain; only 12 of the 17 mean values are in excess of those of the tallest parent line concerned ($\chi^2 = 2{,}88$; $0{,}1 > P > 0{,}05$).

The number of internodes on the stem indicates a tendency towards heterosis, reminiscent of the behaviour of the height values although not so pronounced, while other characteristics appear to have

TABLE 1. *Morphological development and time of flowering of*

Lines, cross combinations and generations	Height of stem				Length of branches				Number of internodes		
	$\bar{x}$	in % of the parents (*cf.* text)	in % of the highest parent line	*n*	$\bar{x}$	in % of the parents (*cf.* text)	in % of the highest parent line	*n*	$\bar{x}$	in % of the parents (*cf.* text)	*n*
1944—1											
*p*1	30,7			13	21,0			22	11,77		13
*p*2	31,1			14	20,9			26	11,93		14
*p*26	26,4			13	21,2			21	12,46		13
*p*29	24,3			14	16,8			25	12,00		14
*p*1 × *p*2	*36,3*	117	117	26	19,3	92	92	51	*12,77*	108	26
*p*1 × *p*26	*34,0*	119	111	20	21,0	100	99	40	*13,00*	107	20
*p*1 × *p*29	30,1	109	98	10	18,6	98	89	20	*12,10*	102	10
*p*26 × *p*29	*28,0*	110	106	6	18,2	96	86	11	12,33	101	6
1944—2											
*p*1	30,9			8	17,9			11	14,00		8
*p*2	34,0			8	22,0			13	15,38		8
*p*25	21,9			10	18,7			18	15,09		11
*p*26	26,1			8	23,7			15	16,50		8
*p*29	27,3			10	24,2			15	15,30		10
*p*31	24,1			8	14,9			12	14,10		10
*p*1 × *p*26	*33,4*	117	108	13	*28,9*	139	122	26	16,39	107	14
*p*1 × *p*29	*32,5*	112	105	13	*26,3*	125	109	24	*15,69*	107	13
*p*1 × *p*31	*33,3*	121	108	11	*28,4*	173	159	21	*15,27*	109	11
*p*2 × *p*25	32,6	116	96	14	*26,0*	127	118	27	15,36	101	14
*p*2 × *p*26	32,0	106	94	12	*23,8*	104	100	24	15,67	98	12
*p*25 × *p*26	*28,5*	119	109	13	*27,4*	129	116	21	15,92	101	13
*p*26 × *p*29	*27,9*	104	102	8	*28,2*	118	117	16	15,63	98	8
*p*26 × *p*31	*26,6*	106	102	12	*25,9*	134	109	28	16,15	106	13
1945—1											
*p*2	33,6			13	16,4			25	11,62		13
*p*25	24,6			14	15,0			16	11,50		14
*p*26	27,2			15	14,9			17	12,20		15
*p*29	25,8			14	15,0			21	11,46		13
*p*31	24,9			13	18,4			20	12,69		13
*p*2 × *p*26	32,9	108	98	13	*22,9*	146	140	23	*12,31*	103	13
*p*25 × *p*26	*30,0*	116	110	12	*22,7*	151	151	19	*12,25*	103	12
*p*29 × *p*31	*29,8*	117	116	9	*22,8*	137	124	16	12,44	103	9
1945—2											
*p*26	35,8			8	—				—		
*p*29	24,6			8	—				—		
*p*53	26,8			10	—				—		
*p*26 × *p*53	32,4	104	91	10	—				—		
*p*29 × *p*53	*27,4*	107	102	8	—				—		

pure lines of L. purpureum and their F_1 hybrids (cf. the text).

Length of leaves			Breadth of leaves			Degree of leaf-indentation			Time of flowering		
$\bar{x}$	in % of the parents (*cf.* text)	*n*	$\bar{x}$	in % of the parents (*cf.* text)	*n*	$\bar{x}$	in % of the parents (*cf.* text)	*n*	$\bar{x}$	diff. (*cf.* text)	*n*
4,19		17	3,54		17	18,76		17	—		
3,77		23	3,34		23	19,69		26	—		
3,13		26	2,72		26	19,58		26	—		
3,80		28	3,15		28	16,29		28	—		
4,21	106	48	3,44	100	48	19,61	102	49	—		
3,51	96	34	2,97	95	34	*21,18*	110	38	—		
3,65	91	11	3,09	92	11	16,61	95	18	—		
3,56	103	11	2,98	101	11	17,42	97	12	—		
5,54		14	4,49		14	19,38		13	15,3		9
4,87		14	3,84		14	19,50		10	13,8		10
4,96		18	3,93		18	17,94		18	12,2		11
4,31		14	3,65		14	21,50		10	11,0		10
5,07		20	4,04		20	16,81		16	6,8		12
4,02		22	3,11		22	23,76		21	23,8		8
4,94	100	25	3,99	98	25	*24,11*	118	19	13,7	+0,5	14
4,74	89	24	3,76	88	24	*20,00*	110	11	7,4	—3,7	13
5,02	105	20	4,08	107	20	*24,57*	114	14	*13,1*	—6,5	11
4,95	101	26	*4,05*	104	26	*20,05*	107	19	*9,4*	—3,6	14
4,50	98	19	3,74	100	19	*25,00*	122	14	14,2	+1,8	14
4,72	102	23	*4,21*	111	23	20,56	104	16	*5,7*	—5,9	13
4,28	91	17	3,79	98	17	20,17	105	6	*0,6*	—8,3	9
4,37	105	27	3,39	97	27	*27,10*	120	20	18,2	+0,8	13
5,22		24	3,80		24	18,87		23	7,9		13
4,59		25	3,44		25	16,60		25	4,0		14
4,41		30	2,97		30	19,39		31	8,0		15
4,71		28	3,05		28	14,89		28	4,4		14
4,50		25	3,06		25	22,20		25	8,1		14
5,49	114	25	3,51	104	25	*20,68*	108	25	*1,6*	—6,4	13
4,90	109	15	3,18	99	15	18,67	104	15	*0,5*	—5,5	12
5,59	121	18	*3,74*	122	18	18,89	102	18	*2,3*	—4,0	9
—			—			—			14,7		8
—			—			—			6,3		8
—			—			—			3,6		10
—			—			—			5,0	—4,2	12
—			—			—			*3,1*	—1,9	8

been influenced, to a higher degree, by seasonal variations. This is particularly apparent in the values relating to the length of branch. The mean values of all the 8 hybrid combinations of 1944—2, and for the 3 combinations of 1945—1, are higher than those of the largest parent line concerned, while, contrariwise, this is not the case with any of the combinations of 1944—1. Since 3 of the hybrids of 1944—1 were cultivated during 1944—2 too, seasonal variations must be suspected to have caused the divergence in behaviour.

The differences in the degree of leaf indentation between different generations are reminiscent of those in growth of the branches. During 1944—1, the relative values of the hybrids are equally distributed around 100, while, in 1944—2, and also in 1945—1, they are all > 100. The probability of such a behaviour in 1944—2, instead of a repeated equal distribution around 100, is 1:256, *i. e.* $\left(\frac{1}{0{,}5^8}\right)$; thus, the hybrids were then characterized by a fairly significant tendency towards a more rich leaf indentation than the average for the parent lines. When discussing corresponding conditions in *L. amplexicaule* (BERNSTRÖM, 1952), it was pointed out that the leaves when developed are incapable of further growth; in addition, the number of teeth is probably determined at an early stage in leaf development. Consequently, the tendency referred to above appeared fairly early in the development of the plants as the leaves studied usually constitute the 3rd or 4th pair of leaves, the plants being only a few cm in height by the time they are formed. Evidently, differences occur between the generations of *L. purpureum* as regards the extent of heterosis, or tendency to heterosis, in the same way as was found in *L. amplexicaule* (BERNSTRÖM, 1952); as was then pointed out, this is a phenomenon frequently observed in experimental materials.

It is noteworthy that, with a greater number of teeth in comparison with one or both of the parent lines, does not also follow a greater size of leaf during 1944—2, as would appear to be a natural expectation. Both with regard to length and breadth, the leaves are about the same size as the average of the parent lines.

Generally, the hybrids are earlier to flower than the respective parental average, although the accelerated rate of development is not so pronounced as in *L. amplexicaule* (*cf.* BERNSTRÖM, 1952). Towards the end of 1945—1 it was found, too, that the hybrids were somewhat earlier to wither than the parent lines. This difference was less obvious during the autumn generations.

One might perhaps be led to suppose that the demonstrated vegetative superiority of the hybrids be a consequence of the differences in the way of origination between the seed from cross-pollination and the seed produced after self-fertilization. The seed from cross-pollination might be argued to have received superior nutrition to that of the pure lines, which would favour the development of the hybrids in a similar manner to that alleged by ASHBY (1937), as regards the appearance of heterosis, although in *Lamium* then only as a result of superior nutrition. Against this, objections may, however, be raised. For one thing, the occasional pure line plants which appeared among the hybrids in consequence of unsuccessful crossing, always corresponded faithfully to the mother line in size, and were often strikingly smaller than the neighbouring hybrids. Above all, it has been found, as will be further related below, that there is no trace of heterosis in intraspecific hybrids of *L. intermedium* and only to a very limited degree in those of *L. hybridum*; in the latter case, it concerns such an unexpected feature as the seed-setting capacity of the flowers (BERNSTRÖM, 1950 a). The hybrids within all the species investigated were produced by the same crossing technique, and it has been shown earlier (BERNSTRÖM, 1952; Table 1) that the percentage of seed-setting in *L. intermedium* and *L. hybridum* was not quite as high as that of *L. purpureum* and *L. amplexicaule*. Thus, supposing that heterosis and tendency to heterosis were caused by the proportion of nutrition of the seed, the prerequisites for heterosis in the former species would be fully comparable to those in the latter species.

The existence of heterosis, and tendency thereto, in *L. purpureum* thus established is of interest, *i. a.*, because it shows that the analogous conditions in *L. amplexicaule* (*cf.* BERNSTRÖM, 1952) are not an isolated phenomenon in the genus. There are several circumstances indicating that the development of *L. amplexicaule* has been characterized by occasional inter- as well as intraspecific hybridization (*cf.* BERNSTRÖM, 1952, and below); then, one may easily presume the common existence of heterosis within this species, however strictly autogamous, to be caused by those genes or groups of genes from other species, which, having entered into *L. amplexicaule*, have been able to stabilize in the pure lines. On the other hand, there are no characteristics in *L. purpureum* indicating an analogous occurrence of interspecific hybridization in its development. It is not possible, however, entirely to leave out of consideration the possibility of such hybridization having occurred; and in any case one must reckon that spontaneous interlinear

hybridization has enabled genic recombinations within the species. This may have been a contributory cause to the discernible ecological differentiation of the species (*cf.* p. 382, and the discussion below).

MÜNTZING (1945) points out that hybrid vigour in F_1 hybrids within strictly autogamous species has been but little reported, and that *Galeopsis Tetrahit* is possibly the first wild species in which heterosis has been observed in hybrids between pure lines. This signifies, however, that such species as, *e. g.*, *Digitalis purpurea* and *Mimulus luteus* are regarded as cultivated plants (ENGLEDOW and PAL, 1934). *Epilobium alpinum* should, however, probably also be regarded as an autogamous species with intraspecific interlinear heterosis, since, according to LOEW (1894) and to some extent also v. KIRCHNER (1911), it is pronouncedly autogamous. PIRSCHLE (1942) shows that hybrids between two lines of *Epilobium alpinum* from Central Europe (supplied by F. VON WETTSTEIN) are characterized by significant heterosis in size of leaf, length of branch, fresh weight and dry weight. If heterosis has only been rarely found in intraspecific hybrids of wild autogamous species the reason may, at least to some extent, be that cultivated plants have generally been chosen for investigations of this kind, and in these heterosis is not unusual in the autogamous ones.

As will be seen from reviews by WHALEY (1944), SMITH (1944), MATHER (1943), and others, of partly diverging opinions, the causes of heterosis are not yet established but still admit of various interpretations. WHALEY, among others, points out that according to the general opinion that heterosis is essentially caused by the suppression, through dominance, of genes unfavourable for the plant development, it is not to be expected that heterosis should be as common in autogamous species as in allogamous ones. In self-fertilizers, unfavourable genes are much more efficiently kept under control by selection than in cross-fertilizers, and the more rigorous the autogamy, the stronger the effect of selection. In strictly autogamous species, therefore, only genes of slightly arresting effect on development are to be expected and, further, in far smaller numbers than they are known to exist in the cross-fertilizers. As the degree of heterosis, broadly speaking, depends on the extent and strength of deleterious genes (WHALEY, 1944), only slight degrees of heterosis can be expected in purely self-fertilizing species.

As shown by the segregations in F_2 from intraspecific hybrids within *L. purpureum*, described below, genic divergencies do however exist between the lines, which are great enough to cause sterility and dwarfishness. Very few F_2 generations of *L. purpureum* have been

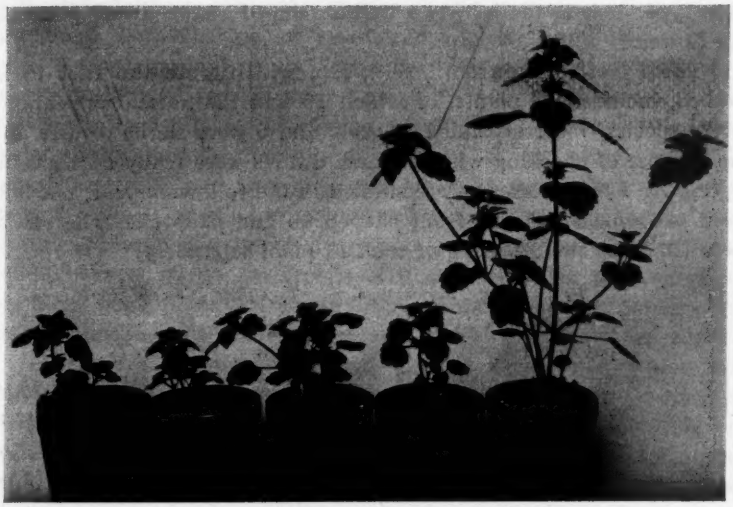


Fig. 1. *L. purpureum*. F_2 generation plants from $p26 \times p29b$. (*Cf.* the text below.)

cultivated, limit of space being one reason, and the other the fact that the greatest interest was focused on the fertility of the F_1 hybrids. In order to investigate the cause of the dwarfishness of the mutants segregated from *p*29 (*cf.* above), which were termed *p*29b, F_2 from $p26 \times p29b$ was, however, studied. The F_1 hybrids were fully vital and fertile. In F_2, on the other hand, apart from small plants of the same type as *p*29b, weak, dwarfish plants of a different type also appeared. Their most characteristic feature was complete sterility, both on the male and female side. In consequence, probably, of the sterility, the flowers remained much longer than the usual time, which is about two days. Habitually, the sterile dwarfs reminded of the dwarf-hybrids between *L. hybridum* and tetraploid *L. purpureum*, preliminarily described earlier (BERNSTRÖM, 1944); common features were a more irregular branching than normal, a more clearly defined nervation of the leaves, and a slow rate of development. Some of the sterile dwarfs obtained in this generation and in a subsequent one did not flower, but died gradually. The dwarf plants of the *p*29b type corresponded habitually far more closely to the normal appearance of the species than did the sterile dwarfs; this is shown in Fig. 1, representing, to the right, a normal *L. purpureum* plant, in the centre two plants of the

*p*29b type, and, to the left, two well-developed sterile dwarf plants in full flower.

The F_2 offspring from *p*26×*p*29b, which were the first to be studied, included 28 plants, of which 16 had the normal appearance of the species, 7 corresponded to *p*29b, and 5 were sterile dwarfs. For the purpose of checking whether these dwarfs were really intraspecific segregates, F_2 generations were studied in the following year, not only from *p*26×*p*29b but also from *p*54×*p*29b and *p*26×*p*54. The results were (showing the offspring of each F_1 plant separately):

F_2	Normal type	Type *p*29b	Sterile dwarfs
*p*26×*p*29b	38	5	—
*p*54×*p*29b	19	8	—
	12	4	11
Total	31	12	11
*p*26×*p*54	32	—	—
*p*54×*p*26	35	—	—

Thus, the sterile dwarfs are also segregated from hybrids between *p*54×*p*29b. However, the divergencies are remarkably great as regards the distribution of fertile (*i. e.* normal plants+the *p*29b type) and sterile plants between the plant progenies of both kinds of hybrids and are, in fact, statistically significant. Thus, χ^2 for the difference between the distributions 23 : 5 and 43: 0, respectively, from *p*26×*p*29b, is $8{,}_{26}$ ($0{,}_{01} > P > 0{,}_{001}$); and for the difference between 27 : 0 and 16 : 11, respectively, from *p*54×*p*29b, χ^2 is $13{,}_{81}$ ($P < 0{,}_{001}$). On the other hand, the distributions of the normal type and the *p*29b type in the two plant offspring within each cross-combination correspond, although χ^2 for the difference between the two distributions from *p*26×*p*29b is as great as $3{,}_{56}$ ($0{,}_{1} > P > 0{,}_{05}$).

Under these conditions, attempts to establish the kind of segregation must be put off until more extensive experiments planned have been conducted. In all the F_2 generations only the features characteristic of the respective parent lines have segregated, mistakes thus being out of question. The differences in segregation between the plant offspring may, for the present, be taken as manifestations of one kind or another of low vitality of the sterile dwarfs.

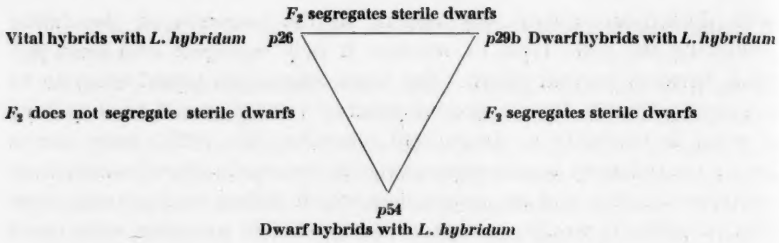
The sterile dwarfs are of interest because they reveal the existence of more important genic differences between the lines than could be expected from the fully vital and fully fertile F_1 hybrids between the lines. It is at present impossible to say whether sterility combined

with dwarfishness can only appear in the presence of the factor producing the *p*29b type, or whether it may segregate also from hybrids between normal plants. The latter alternative would seem to be the most probable, from a general point of view; it would be surprising if what is evidently a chlorophyll mutation like *p*29b, were also a factor contributory to the appearance of the sterile dwarfs, with their complete sterility and an appearance which differs so markedly from that of *p*29b. It would seem more reasonable to presume, as a cause for the appearance of the sterile dwarfs, some kind of complementary action between genes, or groups of genes, which in themselves are harmless; consequently, it would be by pure coincidence that this condition happened to appear in a crossing of which *p*29b formed part.

There are instances, however, *e. g.* in *Drosophila*, which may exemplify either presumption, although, in *Drosophila*, it is a question of lethal effects. Thus, double homozygotes for the genes purple and eyeless are lethal, while the single homozygotes are vital (HADORN, 1951, from CLEMENTE). Furthermore, DOBZHANSKY (1946) has shown on material from *Drosophila pseudo-obscura* that crossing-over between particular chromosomes which are harmless as homozygous, creates new types of chromosomes with lethal and semilethal effects. These effects are presumed to be caused by unfavourable co-operation between genes in the differently composed gene complexes of which either chromosome is supposed to consist.

Whatever the explanation may be to the sterile dwarfs in *L. purpureum*, they constitute a concrete example of two lines having each its own genic balance. Considering the fact that GUSTAFSSON, and others, have shown the existence of heterosis in hybrids of barley which were heterozygous for one or two lethal factors, it is not unreasonable to suppose that F_1 hybrids in *L. purpureum*, which probably carry complementary genes or gene complexes for sublethal dwarfishness, *etc.*, may also be characterized by heterosis (GUSTAFSSON, 1947; GUSTAFSSON, NYBOM and v. WETTSTEIN, 1950).

It has been stated elsewhere that the autotetraploids of some *L. purpureum* lines form vital F_1 hybrids when crossed with *L. hybridum*, while others form dwarfish hybrids of low vitality (BERNSTRÖM, 1949). Habitually, these resemble fairly closely the sterile diploid intraspecific dwarfs referred to above; obviously, however, the same genes cannot cause both kinds of dwarfish growth, as will appear from the diagram below:


F_2 segregates sterile dwarfs
Vital hybrids with *L. hybridum*
*p*26
*p*29b Dwarf hybrids with *L. hybridum*
F_2 does not segregate sterile dwarfs
F_2 segregates sterile dwarfs
*p*54
Dwarf hybrids with *L. hybridum*

Even taking into consideration the fact that the F_2 generations from *p*26×*p*54 were not so large that they showed their lack of sterile dwarf plants quite convincingly, the segregations in F_2 of *p*29b crossed on the one hand with *p*26, and on the other with *p*54, show that they will be largely identical, despite the fact that *p*26 produces vital hybrids with *L. hybridum*, while *p*54 produces dwarfs. The genetic difference between the two latter lines is, therefore, apparent neither in F_1 nor in F_2 after intraspecific crossing, but only in hybrids with *L. hybridum*. Here, however, is another example of an essential genetic divergency between the lines used which may well be presumed to contribute to the occurrence of heterosis in the intraspecific F_1 hybrids. Measurements were not made on the F_1 generation between *p*26 and *p*54, but regarding the kind of hybrids with *L. hybridum*, *p*1, *p*2 and *p*29 form one group, their tetraploids giving dwarf hybrids with *L. hybridum*, while those of *p*25, *p*26 and *p*53 give vital hybrids.

3. L. HYBRIDUM.

A. MORPHOLOGY AND FERTILITY.

This species is rather variable, although the variations always keep well within the bounds of the appearance representative for the species. There are no types transitional to the other species in North-Western Europe; *cf.* the systematic review (BERNSTRÖM, 1952). Several lines have been cultivated and the following were used in crossing experiments:

*h*18 From Svalöf, Sweden. Tall, fairly short branches, dark coloured flowers, coarse leaf indentation.

*h*19 From Svalöf. Differs from the preceding line, among other things, by its longer branches in comparison to the height of the stalk, more rounded leaves, and smaller and lighter flowers.

*h*20 From Wallis canton, Switzerland. Like the preceding, but more low-growing. The leaves are more regularly 3- instead of double-indented than in any of the other lines. The flowers of this and the following line are the smallest and narrowest in the cultivated material.

*h*21 From Waadt canton, Switzerland. Clearly earlier than the other lines. The leaves unusually deeply double-indented, never 3-indented, and with unusually few leaf teeth.

*h*36 From Gothenburg, Sweden. Together with *h*37 rather like *h*18. The flowers are lighter than those of *h*18.

*h*37 From the province of Gästrikland, Sweden. A late line, like *h*38.

*h*38 From Uppsala, Sweden. Of rather low growth, with short branches.

*h*49 From Lund, Sweden. The pollen is yellow instead of red; as to the pollen colour in *L. hybridum*, compare MÜNTZING (1928).

*h*80 From Rouen, France. Together with *h*89 very different from the other lines by its smaller leaf size, finer leaf indentation, more horizontal position of the branches, and throughout more slender and delicate shape. The development is relatively slow.

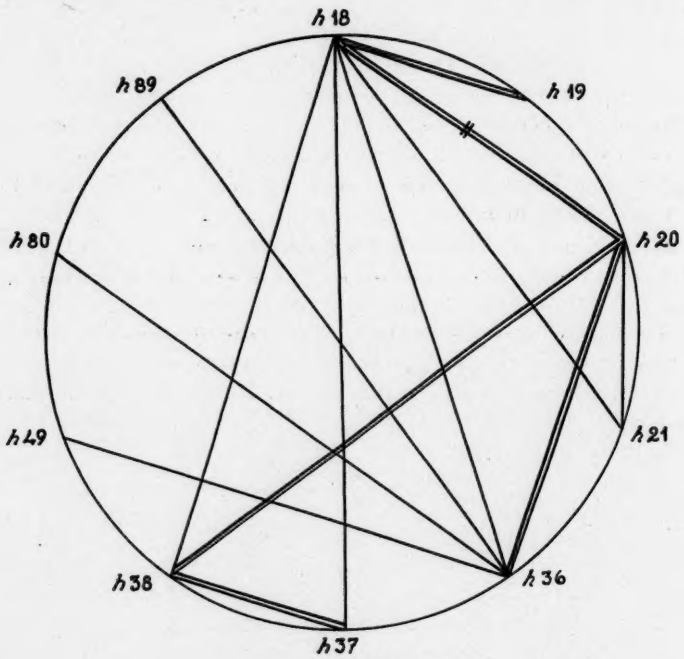
*h*89 From Urbino, Italy. Very like the preceding line.

The chromosome number of all the above-mentioned lines was found to be $2n=36$.

All the cultivated lines of *L. hybridum* were found to be summer-annual. Nor does MÜNTZING (1932 b) know of any lines heritably disposed towards winter-annuality. In this respect, the species appears to be more homogeneous than *L. purpureum* and *L. amplexicaule*, though, as shown above, there is a marked morphological variability. However, judging from the variation between lines cultivated tentatively as well as between those used in crossing experiments, *L. hybridum* does not appear to be quite as variable as *L. purpureum*, which it resembles fairly closely, and from which it probably originates (*cf.* below).

The variations in size of flower are worth noting. The size of flower varies not only between the lines, but also in a high degree with the seasons, and to a different extent in different lines. In the middle of June, the flowers of, *e. g.*, *h*20 and *h*21 measure 15—16 mm, and those of *h*37 and *h*38, 20—21 mm; in the beginning of October, however, in the height of flowering of the second generation, the flowers are smaller, and those lines where the flowers are the smallest in the summer, are characterized by the proportionally greatest re-

duction. Thus, the flowers of *h*20 and *h*21 measure 6—7 mm (*i. e.* about 40 % of the summer length), while those of *h*37 and *h*38 are 15—17 mm (*i. e.* about 80 %). Particularly in lines with small flowers, these are just as often, or more often, closed as open, when autumn flowering has been in progress for some time; especially the helmet becomes strongly stunted, as in cleistogamic flowers of *L. amplexi-*



Scheme 2. (See the text.)

caule. — In none of the other species studied exist so great differences between the lines as regards the length and size of the flowers, as in *L. hybridum.*

Observations during the course of the experiments indicate that *L. hybridum* differs from the other species in being clearly more sensitive to changes in the soil, position, *etc.* Thus, the phenotypical variation within the lines is clearly greater in this species than in the others, and is particularly apparent with reference to height of plant and size of leaf.

Interlinear hybrids were produced as shown in Scheme 2 (concerning its interpretation, *cf.* p. 384).

The purpose with these hybrids was primarily the same as that of the interlinear hybrids in *L. amplexicaule* and *L. purpureum*, *i. e.* to test the possible existence of cytological or other differences between the lines, which might reduce the fertility of the hybrids, and which — as far as cytological divergencies are concerned — might well be expected by reason of the allotetraploid nature and the variability of this species (*cf.* below). The hybrids were also measured in the same way as those in the diploid species, with a view to checking the possible occurrence of heterosis. The number of plants in each hybrid combination will be seen in Table 2, representing the seed-setting. No clear morphological differences were found between reciprocal crossings.

The measurements show that there is no general tendency in this species towards a stronger vegetative development in the hybrids than in the parent lines. In almost all the characters measured the relative values (*i. e.*, the mean value of the hybrids in percentage of the common mean value of the parent lines), proved to exceed and to fall short of 100 by almost equal degrees; only the relative values of the number of leaf teeth are all 100. Although, unfortunately, the numbers of individuals of the hybrids were often low, this should not have influenced the results if there were really any differences. Nor did the hybrids by visual examination, in contrast to those of the two diploid species (*cf.* also BERNSTRÖM, 1952), give the impression of superior vegetative development in comparison to the pure lines; therefore, as far as the present experimental results indicate, *L. hybridum* differs markedly in this respect from the diploid species.

This condition is difficult to explain. MÜNTZING (1945) has shown that heterosis may generally characterize interlinear hybrids of an autogamous tetraploid species such as *Galeopsis Tetrahit*. From a genetical point of view, there is no reason to expect tetraploids to be characterized by a less pronounced vegetative development than diploids, supposing that the degree of heterozygosity is the same in both cases. The genic variation within *L. hybridum* does not seem to be much inferior to that of the diploid species studied, as judged from the interlinear variation. With the present material it is difficult to state what genetical differences may have caused the dissimilar behaviour of the diploid and the tetraploid *Lamium* species, respectively.

The pollen fertility in *L. hybridum* is very good. It was examined in 1—7 generations of all lines used for intraspecific crossings, the

TABLE 2. *Seed-setting in pure lines of L. hybridum and in their F_1 hybrids (cf. the text).*

Generation	1944—2			1945—1			1945—2			1946		1948			1949		
Lines and hybrids	$\bar{x}$	rel. value	*n*	$\bar{x}$	rel. value	*n*	$\bar{x}$	rel. value	*n*	$\bar{x}$	*n*	$\bar{x}$	rel. value	*n*	$\bar{x}$	rel. value	*n*
								Pure lines									
*h*18	82,2		8	48,5		14	76,8		18	62,1	7						
*h*19	84,6		6	82,2		14	81,3		13	89,0	8						
*h*20	91,3		11	85,8		14	79,2		8								
*h*21	94,4		8	89,7		14	94,8		6			70,0		14	90,3		14
*h*36	79,2		8	80,9		14	70,3		10	85,3	16	80,7		13	76,5		14
*h*37	80,0		11	82,1		14				82,2	8	81,4		14	81,0		16
*h*38	87,7		11				81,9		9								
*h*49															85,9		7
*h*80												80,5		12			
*h*89												78,4		14	80,8		7
								F_1 hybrids									
*h*18 × *h*19	85,7	103	6														
*h*18 × *h*20	89,7	103	4	83,4	124	9	88,5	113	9								
*h*18 × *h*21							87,3	102	12								
*h*18 × *h*36							83,9	114	12								
*h*18 × *h*37				81,1	124	8											
*h*18 × *h*38							90,7	114	11								
*h*20 × *h*21	88,6	95	2														
*h*20 × *h*36				82,5	99	15											
*h*20 × *h*38	87,5	97	6														
*h*36 × *h*49															83,5	103	10
*h*36 × *h*80												79,6	99	13			
*h*36 × *h*89												77,8	98	14			
*h*37 × *h*38	87,3	104	10														

mean values of line and generation varying between 92,6 and 99,5 % of good pollen grains. Only 3 mean values out of a total of 32 are, however, < 96; the average of all mean values for pure lines is 97,8. Plants with a pollen fertility of less than 90 % do not even amount to 0,5 % of all investigated.

The pollen fertility of the intraspecific hybrids is throughout as high as that of the parent lines. Their mean values in per cent of the common mean value of the parents lie between 99 and 103; most of the relative numbers are $= 100$, and the others are evenly distributed around this figure. Thus, as far as the lines investigated are concerned, there is no interlinear partial sterility whatever in this species.

The seed-setting in *L. hybridum* has been briefly dealt with earlier (BERNSTRÖM, 1950 a). It is not as high as might have been expected, bearing in mind the pollen fertility, and the seed-setting in *L. purpureum* and *L. amplexicaule*. It is, however, generally good (*cf.* Table 2). *h*20 and *h*21 differ somewhat from the other lines in having, as a rule, achieved the best seed-setting; even during 1947 (not included in Table 2), when only *h*21 and *h*37 were studied, *h*21 was the best line with a seed-setting of 92,4 % as against 79,1 % in *h*37. A connection may be presumed between the conspicuously good seed-setting of the 2 Swiss lines and their outstanding tendency, referred to above, towards closed flowering, the latter possibly rendering self-pollination more certain (*cf.* BERNSTRÖM, 1950 b, 1952).

When in separate lines the seed-setting is occasionally reduced considerably below the average, which appears to occur most easily in *h*18, this is, in most cases, caused by external conditions of one kind or another, in the first place probably by difficulties in self-pollination. To mention a few striking examples of reduced seed fertility, the seed-setting of the plants in *h*18—45—1 varied between 20 and 83 %, in *h*18—46 between 44 and 80 %, and in *h*36—45—2 between 47 and 82 %. A number of the plants in *h*36—45—2 showed scars from attacks by aphides, which, in this case, may have caused the reduction in fertility. Evidence that the reduction in the seed fertility is caused by external conditions will be found in the fact that the pollen fertility, when examined, never decreases simultaneously with the seed-setting; this would have been expected if the reduction in the seed fertility were genetically conditioned. From the plant with the lowest seed fertility in *h*18 during 1945—1, with 20 % seed-setting, an offspring containing 6 plants with an average seed-setting of 78,3 % were cultivated in 1945—2. Similarly, 12 individuals from a plant of the same

line, showing 83 % seed-setting in 1945—1, had an average seed fertility of 76,1 % in 1945—2. Both offspring groups had an average pollen fertility of 98,5 %.

The seed-setting of the interlinear hybrids has been dealt with earlier (BERNSTRÖM, 1950 a). In connection with Table 2, reference will only be made here to the greater evenness of the seed-setting between hybrid combinations within the generations, than between the pure lines; this depends on the tendency towards improved seed-setting in the hybrids to which attention has been drawn earlier. The average seed-setting in the hybrids, computed on the mean values of the hybrid combinations, is 85,1 %, while the corresponding figure for the pure lines is 81,1 %. Only those lines which entered the hybrids during the same generation are included. However, the figures cannot be regarded as entirely correct, since seasonal variations combined with unequal representation of pure lines and hybrids in the different generations probably have influenced the result.

B. CYTOLOGY AND ANEUPLOIDY.

In *h*36, which is a line frequently used especially for species hybridization, it has been possible to investigate the chromosome pairing in I M. In all, 172 cells from 3 buds were analysed. Bivalents were the only kind of configurations found, and the pairing in bivalents was very good. 2 univalents instead of 1 bivalent were found in only 7 cells, and in no cell were there more than 2 univalents. The cells with unpaired chromosomes were distributed as follows:

Fix.-Nos.	18 II	17 II + 2 I
L 1720 D	84	2
L 1720 B 1	16	1
L 1720 B 3	65	4
Total	165	7

No other stages of meiosis were studied. Fig. 2 shows the 18 bivalents in a cell, drawn separately.

Here it should be mentioned that it has been possible to establish, by means of cytological studies on species hybrids on which will be reported in a later paper, that *L. hybridum* is an allopolyploid. Probably, *L. purpureum* is one of the parent species. Below will be dealt more in detail with the question about its origin.

In order to test whether and, if so, to what extent aneuploid plants appear within the species, each cultivated plant was examined with a view to discerning some features which might indicate a divergence in the chromosome number; attention was also paid to reduced seed-setting. The unusually wide phenotypic variability of *L. hybridum*, referred to above, rendered this investigation difficult. Of 7 plants cytologically examined for different reasons, all had $2n=36$, except 1 plant from *h*21—45—2, which had $2n=35$. This plant was investigated because its seed-setting was only 53 %, while 6 sister plants had between 91 and 98 %. (The 35-chromosome plant is not included in the mean value for *h*21 in Table 2.) Unfortunately, pollen samples could not be taken from any plant of *h*21 during this generation, because the flowers were closed, containing very small quantities of pollen. There was nothing in the outward appearance of the 35-chromosome plant to indicate its aneuploid nature; the examination was made difficult, however, by the fact that the lowest leaves of the inflorescence on the stem which, in the *Lamium* species, are the most delicate indicator of the genetic constitution, were here deformed through the activity of insects, and therefore of no value. The size of the 35-chromosome plant was the same as that of its sister plants.

Thus, aneuploid plants appear spontaneously in *L. hybridum*, although they are evidently rare. However, a small number of aneuploid plants were artificially produced, their chromosome numbers varying between $2n=33$ and $2n=38$. In most cases, they differed from the normal appearance in various ways. Their seed-setting as well as their pollen fertility were always weakened to a rather significant degree, and this was also the case with the 35-chromosome plants. A more detailed account of the aneuploids will be given in a later paper; here they are mentioned in order to show that such plants are perfectly viable — even a 33-chromosome one — and that the sparse occurrence of spontaneously originated aneuploids as well as the absence of more extreme aneuploids may depend, essentially, on the regularity of meiosis.

4. L. INTERMEDIUM.

A. MORPHOLOGY AND FERTILITY.

Of this species, the following 8 lines from various places were used in crossing experiments:

*i*22 From Svalöf, Sweden.
*i*23 From Svalöf.

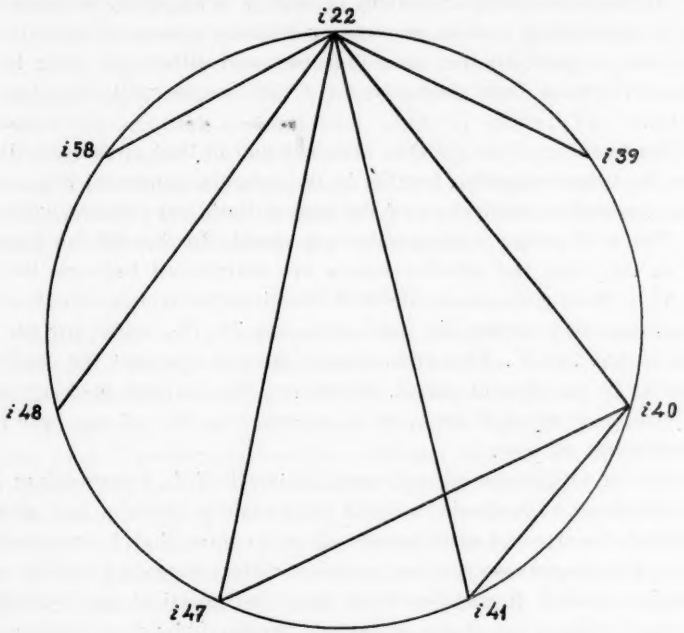
*i*39 From the neighbourhood of Gothenburg, Sweden.
*i*40 From the province of Värmland, Sweden.
*i*41 From the central region of the province of Bohuslän, Sweden.
*i*47 From the southernmost point of the Isle of Öland, Sweden.
*i*48 From the island of Grötö, Southern Bohuslän, Sweden.
*i*58 From Pauroinkylä, Finland.

Despite the fact that lines from so many and in some cases so widely separated places, were brought together to represent this species — which is evidently, within its distribution area, generally more sparingly met with and more sparsely growing than the other species studied within their areas — it was found that there is a very great similarity between the lines. The similarity is, in fact, such that not a single one of them differs from the others in any clear characteristic. However, there are possibly minor differences between them; if so, they do not appear in each generation. Thus, *i*47, and possibly *i*48, may have slightly smaller leaves than the other lines; *i*47 may also be slightly earlier than the others. The differences are, however, doubtful; as a rule, lack of space prevented cultivation of all lines in the same generation, or cultivation of so many plants of each line that more definite comparisons could be made. Disregarding these difficulties it is, however, obvious that *L. intermedium* is morphologically and physiologically very homogeneous. Like *L. hybridum*, it is always summer-annual. In 6 of the 8 lines, the chromosome number was found to be $2n=36$.

The fertility of *L. intermedium* is good. In pure lines, the mean values per generation of the pollen fertility, which was to a varying extent examined in 5 consecutive years, varied between 95,2 % and 99,2 %. The average of all mean values of the pollen fertility (these are 25, and based on a total of 270 plants) is 97,7 %. The pollen fertility of individual plants is almost always considerably more than 90 %; the occasional exceptions will be dealt with later.

The seed-setting in *L. intermedium* amounts to only about 70 % of that which is numerically possible; in this, it resembles the seed-setting in *L. hybridum* which amounts to about 80 %. Thus, the averages of the seed-setting in the *L. intermedium* lines varied between 48,0 and 84,3 %; the mean value of all averages (these are 25, and distributed over 8 generations) is 71,0 %. The degree of seed-setting is conditioned by the seasonal variations, but not by the time of year; and no line differs from the others in presenting a lower or higher

seed-setting than the rest. Both the seed-setting and the pollen fertility were studied in all lines except *i*23, during 2—6 generations. The seed fertility of single plants may occasionally be as low as about 35 %, but if this reduction is not cytologically caused (*cf.* below), the pollen fertility is always very high, and the offspring characterized by the



Scheme 3. (See the text.)

fertility representative for the species. Repeated experiments have confirmed this.

As in the other species studied, interlinear F_1 hybrids were also produced in *L. intermedium* with a view, especially, to tracing possible cytological divergences between them. The interlinear hybrids are indicated by Scheme 3.

No reciprocal hybrids were produced. The hybrids were cultivated during 4 different generations, with 1—3 hybrid combinations to each. Every hybrid combination consisted of 6—14 plants.

The hybrids did not show that tendency towards greater growth than that of the parents which was typical of the interlinear hybrids

in *L. amplexicaule* and *L. purpureum*. In most of the hybrid combinations, together with their parent lines, measures were taken of the height of stem, the number of internodes, the length and breadth of leaf, and the number of leaf indentations; the commencement of flowering was also registered. However, it has been impossible to trace any clear differences, either numerical or visibly perceptible, between any hybrid combination and its parents, or between groups of hybrids and pure lines, respectively, no more than any real differences were found between the pure lines themselves. — This matter will be explained directly.

The fertility of the hybrids, also, is equal to that of the pure lines. Thus, the values of pollen fertility in the hybrids, expressed in per cent of the common mean values of the parent lines, vary between 97 and 101. The seed-setting corresponds very closely to that of the parents, too; in this case the relative values are distributed between 86 and 106. As to those generations in which interlinear hybrids were cultivated, the average seed-setting for pure lines was 76,2 %, while, for the hybrids, it was 73,5 %. Thus, this species does not present the condition found in *L. purpureum* and *L. hybridum*, viz. that the seed-setting of the interlinear hybrids tends to be superior to that of the pure lines (BERNSTRÖM, 1950 a).

The investigations of cultivated material of *L. intermedium* and, to some extent, of herbaria material from various quarters, and also the results of the crossing experiments, all go to show that *L. intermedium* is a very homogeneous species. In this it differs sharply from the other 3 species studied. It may be stated here that genetical and cytological experiences from the study of various species hybrids, as well as the direct synthesis of *L. intermedium*, show that this species is an allotetraploid between *L. purpureum* and *L. amplexicaule*; we will revert in a later paper to the evidence available in this direction. The disclosure of the nature of *L. intermedium* is surprising in view of the impossibility, so far, of hybridizing the wild growing diploid lines of *L. purpureum* and *L. amplexicaule* (MÜNTZING, 1926; JÖRGENSEN, 1927); it is only by cross-pollinating tetraploid *L. purpureum* with diploid *L. amplexicaule* that, after many attempts, hybrids between them could be produced (BERNSTRÖM, 1953).

From the knowledge of the conditions mentioned above it is reasonable to suppose that *L. intermedium* originated on one single occasion and, further, that this occasion was not too distant from a biological point of view, but rather recent enough to have scarcely

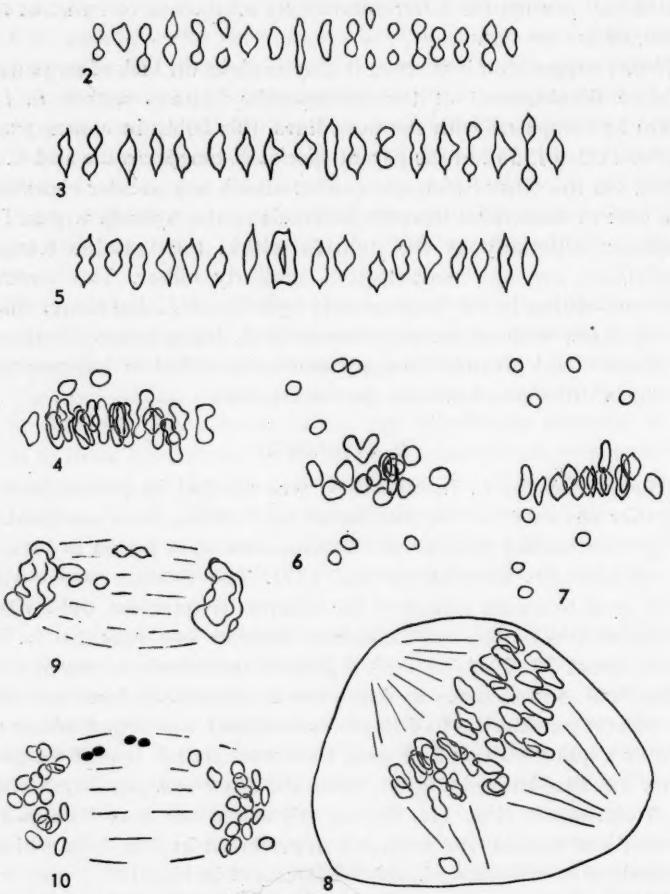
afforded any perceptible differentiation by mutations, or by some other method, of the species.

If this supposition is correct, it may explain the lack of any superior vegetative development of the intraspecific hybrids within *L. intermedium* as compared with the pure lines, this being in contrast to the conditions often found in the parent species *L. amplexicaule* and *L. purpureum*. On the other hand, one cannot attach any greater significance to the lack of tendencies towards heterosis in the hybrids within *L. intermedium*, especially as the hybrids within the variable tetraploid *L. hybridum* are not characterized similarly either. The somewhat better seed-setting in the intraspecific hybrids of *L. hybridum* than in the pure lines, without correspondence in *L. intermedium*, is the only sign which could possibly be explained as an effect of heterozygosity, implying a difference between the two species.

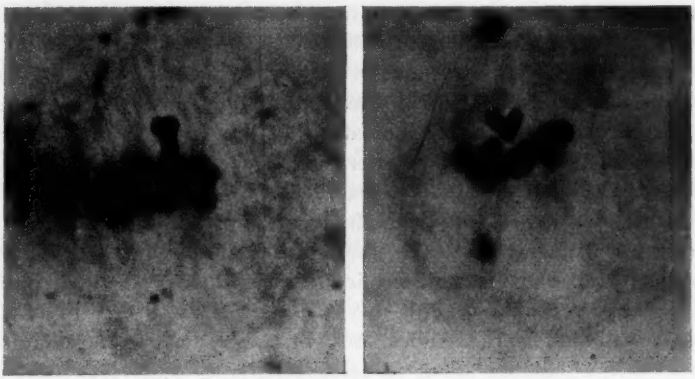
B. CYTOLOGY.

The meiosis in *L. intermedium* was studied in plants from *i*41. Altogether 267 cells in I M, distributed on 8 slides, were analysed. The quite predominating number of chromosomes were joined in bivalents; 66 % of the cells investigated had 18 II. The fixation was not sufficiently good to enable studies of the chiasma frequencies, but 2 chiasmata appeared to be the most common number (see Figs. 3—5). However, in about 20 % of the cells a pair of univalents occurred instead of a bivalent; and as many as 6 univalents occurred in buds with otherwise normal pairing. In 4 of the cells analysed was found either a trivalent or a quadrivalent; 2 of each kind were found. One of the quadrivalents was ring-shaped (Fig. 5), while the other was possibly X-shaped, with 3 chiasmata (Fig. 11). Of the trivalents, one was V-shaped and the other rod-shaped; the former is reproduced in Fig. 6, in which the odd number of univalents is also visible, and in Fig. 12.

I M in one of the buds (No. 7 in Table 3) differed from the others in having a fairly pronounced asyndesis. Cells with as many as 16 to 18 univalents were found. Whatever the number of univalents in a cell, the bivalents were always grouped in an equatorial plate; the univalents were irregularly distributed in the plasm. Table 3 shows the frequencies of cells with different numbers of univalents. The distribution of the univalents in the cells can be seen in Figs. 6—7. The appearance in several cases of 4—6 univalents per cell in slide No. 6 may possibly have been caused by the same factor which produced the stronger asyndesis in slide No. 7.



Figs. 2—10. Meiosis in *L. hybridum* and *L. intermedium*. — Fig. 2, *L. hybridum*; I-M in side view (18 II separately drawn). — Figs. 3—10, *L. intermedium*; Fig. 3, I-M in side view (18 II separately drawn); Fig. 4, I-M with 17 II+2 I; Fig. 5, I-M with 1 IV+16 II. — Figs. 6—7, I-M from a bud with partial asyndesis; Fig. 6, 1 III+ +13 II+7 I; Fig. 7, 14 II+8 I. — Fig. 8, I-M in an exceptional cell with 36 II, probably originated by fusion of two tetraploid cells after pachytene. — Figs. 9—10, I-A; two cells with each a pair of univalents which are stretched-out but probably not dividing.



Figs. 11—12. Meiosis in *L. intermedium*. — Fig. 11, a possibly X-shaped quadrivalent; Fig. 12, I-M with 1 III+5 II+2 I visible in a cell with partial asyndesis (the same as in Fig. 6).

In this material of normal plants from a pure line the cause of the asyndesis will most probably have been of an external nature. Truncation of the plant for the purpose of bud fixations 15 days before the fixation now under discussion may, perhaps, have affected its physiology too much to allow a normal process of meiosis. Slides Nos. 6 and 8 were fixed at the same time and may originate from the same

TABLE 3. *Frequencies of configurations and univalents in I M of L. intermedium.*

Per cell	Slide number								n	n (except slide No. 7)
	1	2	3	4	5	6	7	8		
1 IV + 16 II	1				1				2	2
1 III + 16 II + 1 I				1					1	1
1 III + 13 II + 7 I							1		1	—
18 II	31	39	11	23	27	23	5	18	177	172
17 II + 2 I	11	5	4	10	5	10	4		49	45
16 II + 4 I						4	9		13	4
15 II + 6 I				1		1	7		9	2
14 II + 8 I							6		6	—
13 II + 10 I							5		5	—
12 II + 12 I							2		2	—
10 II + 16 I							1		1	—
9 II + 18 I							1		1	—
Total	43	44	15	35	33	38	41	18	267	226

plant as No. 7. The external conditions were about the same as those at the fixation of the material in slides Nos. 1—5.

In slide No. 6 there was also in the corner of a microsporangium a larger-than-normal cell, which was found to have 36 II; see Fig. 8. The regular bivalent pairing indicates that this cell was originated by the fusion of 2 tetraploid ones after pachytene; evidently this is an instance of syndiploidy (DARLINGTON, 1937).

The slides Nos. 1—4 originate from intact plants and here the appearance of univalents is not likely to have been caused by external influence, particularly since there is almost exclusively a single pair of univalents per cell.

I A was studied in 3 slides. In 2 of these, the chromosomes had separated in altogether about 70 cells, apparently quite regularly. The stages were too late to make it possible to examine the distribution of the chromosomes. In only 1 cell were 2 univalents, seemingly in the process of dividing, but in fact more probably only strongly stretched-out between the anaphase groups (Fig. 9). In the 3rd slide, No. 7 in Table 3, a number of cells contained 1—2 univalents which were also very much stretched-out; a cell with 2 univalents is reproduced in Fig. 10. The existence of cells with a single univalent situated between the poles indicates that univalents also move straight to the poles; this is indicated, too, by the distribution of the chromosomes in Fig. 10, which is 16—18, while that in the adjoining cell was 17—19. The existence of a single cell with apparently dividing univalents among about 70 cells with apparently normal separation in the 2 former slides, indicates that here, also, the univalents move to the poles, without previously taking up a position between the poles. Unfortunately, no satisfactory slides with II A were available for examination; nor was any univalent observed in which the chromatids had clearly separated from each other; it is therefore uncertain and, in fact, unlikely that the univalents divide in I A. Those univalents which have taken up a position between the poles are probably only subjected to an intense stretching which looks like a division (*cf.* BERNSTRÖM, 1952, pp. 198—99).

It was possible to study II M somewhat closer in 2 slides; unfortunately, however, the distribution of the chromosomes could be investigated in 39 PMC's only. In 38 of these, both plates had 18 chromosomes; in the remaining cell there were 18 chromosomes in the one plate, and 17 in the other, while 1 chromosome lay apart in the periphery of the cell. Although of limited value because of its inconsider-

able extent, this investigation indicates that the predominating number of gametes receive 18 chromosomes, despite the fact that about $^1/_5$th of the PMC's in I M had a pair of univalents. It must not, however, be taken for granted that the 18 chromosomes are always those which constitute the genome.

It has been stated above that *L. intermedium* is an allotetraploid between *L. purpureum* and *L. amplexicaule*. It should be added here that hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule* have, as a rule, 9 II+9 I in I M; occasional cells, however, present a trivalent or other divergencies, indicating heterogenetic pairing, although of low frequency (BERNSTRÖM, unpubl.). Certain facts regarding species hybrids with *L. intermedium* and those between *L. purpureum* and *L. amplexicaule* may indicate that *L. intermedium* is not built up of chromosome-structurally pure genomes, but that structural recombinations between the genomes involved have, to some extent, taken place — probably when the species originated. The predominant formation of 18 II in I M does show, however, that the pairing in *L. intermedium*, too, is generally purely homogenetic, and the occurrence of occasional trivalents and quadrivalents may be interpreted, also in this instance, as a consequence of incidental heterogenetic pairing between chromosomes from the two parental species (the terms from WADDINGTON; in STEBBINS, 1947). The scarceness of the multivalents gives support to the presumption that the heterogenetically conjugating chromosomes are only slightly homologous. Since they are able to form multivalents, however, it is obvious that heterogenetic pairing must result in bivalents, too, and this may, in its turn, explain the high frequency of pairs of univalents. If 2 chromosomes which are only partially homologous are able to form a bivalent by heterogenetic pairing, it should follow that the chances of the sister chromosomes of the two parties of also forming a bivalent are smaller than those of the two fully homologous chromosomes not to form a bivalent. The appearance of pairs of univalents may perhaps also be interpreted, to some extent, as simply due to unsuccessful homogenetic bivalent formations.

In the case of *L. hybridum*, in which no multivalents but pairs of univalents were found although of considerably lower frequency than in *L. intermedium*, the same reason for the appearance of univalents as in the case of *L. intermedium* may well be supposed to exist.

It may be of interest, in this connection, to point out that a differ-

ence in the degree of pollen fertility exists between the diploid and the tetraploid species of *Lamium*. As a result of the strong autogamy of these species, they all show a very high pollen fertility. Computation of the mean value of all available mean values of pollen fertility in pure lines, for each species, shows, however, that the diploid species have a higher pollen fertility, as will be seen from the following table:

Species	$\bar{x} \pm m$	Number of mean values
L. purpureum	98,6 ± 0,118	39
L. amplexicaule	99,0 ± 0,098	28
L. hybridum	97,8 ± 0,273	32
L. intermedium	97,7 ± 0,180	25

Although the difference is very small indeed, an analysis of variance as given below gives a quotient between the mean squares for the comparison diploid—tetraploid species, and for »within» of 31,46 ($P < 0{,}001$). Thus, the pollen fertility of the tetraploid species is quite significantly inferior to that of the diploids, despite the fact that the difference between the mean values amounts to only about 1 %.

Cytological irregularities are probably less responsible for the inferiority of the tetraploids; this is more likely caused by the tetraploid nature in itself, because if this were not so, it would have been reasonable to expect in *L. intermedium* a pollen fertility inferior to that of *L. hybridum*, since in the former meiosis is more irregular than in the latter. Disturbances within the genotype, adapted to the diploid state, as well as disturbances of the hereditary balance which is to be found, in any given species, between the genotype and the anatomical structure (such as the size of cells), and the physiological processes, are now fairly generally looked upon as the main cause of that reduction in fertility which is always to a greater or smaller degree a characteristic of new autotetraploids (*cf.*, *e. g.*, STEBBINS, 1950). It is a reasonable presumption that also new allopolyploids should always be characterized to a greater or smaller degree by a corresponding reduction in fertility, which may only be eliminated gradually by various processes. Even if new allopolyploids as well as wild-growing ones are sometimes described as fully fertile, particularly in respect of fertility as judged on the pollen from the proportion of empty grains, the interpretation of the term »fully fertile» may be a matter of degree, as shown, *i. a.*, by the investigation related above.

	df	sd²	var.
L. amplexicaule+*L. purpureum*—*L. hybridum*+*L. intermedium*	1	31,84	
L. amplexicaule—*L. purpureum*	1	1,79	
L. hybridum—*L. intermedium*	1	0,00	
Within species	120	121,39	1,01
Total	123	155,02	

$$\frac{31,84}{1,01}=31,46 \ (P < 0,001)$$

C. ANEUPLOIDY.

About 2 % of the plants investigated in pure lines and line hybrids of *L. intermedium*, *i. e.* 10 plants out of 530 examined, were found to be aberrant, mainly in the appearance of the leaves in connection with reduced fertility. *L. intermedium* is not so susceptible as *L. hybridum* to external factors which influence morphology; and this applies to the shape of leaf, too. An aberrant plant in *L. intermedium* therefore stands out rather clearly. In *Lamium*, the shape of the lowest leaves in the inflorescence in connection with the shape of the calyces is the best systematic characteristic, the shape of leaf being generally also the most ready morphological indicator of irregularities in the chromosomes.

Very often the aberrant features do not stand out clearly before flowering, but only after this has been in progress for some time. Then the root growth is minimal, and since it has proved impossible to make cuttings take root, the chromosome numbers could not be established for all the aberrants found. Attempts to examine meiosis in individual plants within this material also meet with considerable difficulties. There is on each plant a very small number of buds in a suitable stage of development, and the chances of finding among them a bud with well fixed divisions are even smaller. Consequently, it has not been possible to investigate the aberrant plants cytologically as thoroughly as would have been desirable in view of the fact that cytological disturbances obviously give rise to these plants. This is evident both from the chromosome numbers found, and from chromosome numbers and characteristics in progenies from aberrant plants.

Thus, since the investigations have been concerned with the exterior characteristics of the aberrant plants, and with their offspring, rather than with their cytology, a more detailed presentation of the subject is unnecessary. The characteristic features of the aberrant plants found are reviewed in Table 4. The leaves of some of the plants are reproduced in Fig. 13. It will be seen from Table 4 that at least

TABLE 4. *Characteristics of aberrant plants in pure*

	Plant number	2n	Seed fertility	D:o of the sister plants $\bar{x}$	Pollen fertility	D:o of the sister plants $\bar{x}$	Height of stem (cm)	D:o of the sister plants $\bar{x}$
1	*i*40$_6$-44-2	36	50	85,9 n = 8	—	97,8 n = 6	—	—
2	*i*41$_5$-44-2	—	54	81,5 n = 11	—	98,3 n = 8	—	—
3	*i*41$_{13}$-44-2	36	18	»	—	»	—	—
4	*i*39$_6$-45-2	—	48	81,8 n = 11	74	98,9 n = 11	88	81 n = 11
5	*i*41a$_{14}$-45-2	36	33	80,2 n = 17	—	97,9 n = 12	46	85 n = 17
6	*i*104$_7$-45-2 (104 = *i*39 × *i*22)	—	63	74,3 n = 11	—	97,1 n = 10	65	76 n = 11
7	*i*41a$_{21}$-46	35	37	72,1 n = 37	—	—	normal	—
8	*i*41a$_{37}$-46	36	41	»	—	—	lower than normal	—
9	*i*39$_4$-48	37	37	62,9 n = 12	94	99,0 n = 12	normal	—
10	*i*39$_{27}$-48	37	44	»	95	»	»	—

3 of the aberrant plants had aneuploid chromosome numbers, and were, therefore, chromosomally unbalanced; however — as will soon be seen — this lack of balance need not always in itself have caused such divergences in fertility and appearance as have been found. Four of the other plants had $2n=36$, but the offspring from 2 of them verify, more or less clearly, the hereditary unbalance of the mother plants.

lines, and hybrids between lines, of L. intermedium.

Main exterior characteristics	Offspring		
	n	2n	Notes
Leaves aberrant as in $i41_{13}$-44-2, although not so distinctively. Somewhat darker green than normal	6	36	Plants quite normal
Similar to the above	3	36—37	*Cf.* p. 417
Leaves somewhat crumpled, fewer and larger indentations, broader than normal. The calyces somewhat smaller and more closed. Later, and of a darker green	10	36	9 of normal fert.; 1 plant partially sterile. *Cf.* p. 415
Closely resembling the last-mentioned in all aberrant features	38	35—37	*Cf.* Table 5, and p. 417
Weak, a lighter green, but with darker green spots having clear or vague outlines. Simply shaped leaves with sparse indentation. Small, mostly closed flowers	6	36—38	*Cf.* p. 415—17
Leaves aberrant as in $i41_{13}$-44-2, although not so distinctively. The calyces of normal type	—	—	—
Leaves with fewer and larger indentations, broader and more kidney-shaped than normal, but with smooth surface (*cf.* Fig. 13)	23	35—38	*Cf.* Table 6, and p. 418
Leaves of normal shape, but indentation irregular; indentations in part large and rounded (Fig. 13). The calyces sometimes 4-6-indented	—	—	—
Leaves with almost normal indentation (Fig. 13), but somewhat crumpled and stiffer than normal. Late	—	—	—
Leaves with fewer and greater indentations than normal, even more crumpled than in $i39_4$-48 (Fig. 13). The stem, too, with crumpled surface. Sepals shorter than normal. Later than $i39_4$	11	36	*Cf.* p. 420

Thus, the partially sterile plant from $i41_{13}$—44—2 (Table 4, extreme right column), had only 52 % of seed-setting and 89 % of pollen fertility, although it did not present any morphological divergences. Its sister plants had an average seed-setting of 68,8 %, and a pollen fertility of 97,1 %. — The characteristic features of the offspring in 1946 from $i41a_{14}$—45—2 were:

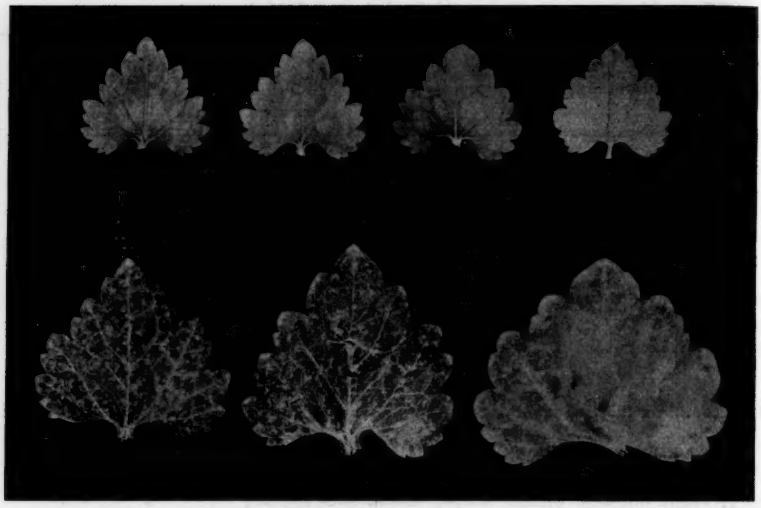


Fig. 13. Leaves from aberrant plants of *L. intermedium*. Upper row from left to right: two leaves of normal appearance, one leaf from each of the plants $i41a_{21}$—46 and $i41a_{37}$—46. Lower row from left to right: one leaf of normal appearance, one leaf from each of the plants $i39_{4}$—48 and $i39_{27}$—48. The differences in size of leaves between the rows are due to seasonal variations.

Plant No.	2*n*	Seed fert.	Pollen fert.	Height of stem	Indentations per leaf	Greatest flower length
*i*41b 1	37	8	47	52	11,75	13—14 mm
2	36	23	81	45	12,50	17
3	38	3	41	50	10,75	14
4	37	81	94	45	21,75	21
5	36	60	93	39	—	20
6	36	80	93	38	17,00	21

The number of indentations per leaf was computed from the 2 lowest pairs of leaves in the inflorescence of the stem. It may be mentioned, by way of comparison, that the seed-setting in pure *i*41 of the same generation, excluding the 2 aberrant plants in Table 4, was on an average 72,1 %, the height of stem 49,9 cm, and the number of indentations per leaf 20,07. The average length of flower in *L. intermedium* is fully 22 mm in summer. The variation in chromosome numbers, fertility, and morphology of the offspring from $i41a_{14}$—45—2, therefore, clearly shows the mother plant to have been cytologically unbalanced despite a chromosome number of 36. It may be added that

TABLE 5. *Properties of euploid and aneuploid plants in the offspring from i39$_6$—45—2.*

Character	2n = 35		2n = 36		2n = 37		t-value for the diff.
	x̄ ± m	n	x̄ ± m	n	x̄ ± m	n	35—36
Seed-setting	26,7 ± 2,20	27	70,4 ± 1,07	8	67,5 ± 4,75	3	***
Pollen fertility	79,1 ± 1,65	27	97,9 ± 0,39	8	97,5 ± 0,90	3	***
Height (cm)	42,4 ± 0,59	27	47,1 ± 0,74	8	45,7 ± 1,25	3	4,9***
Number of leaf-teeth	15,80 ± 0,308	23[1]	19,34 ± 0,450	8	19,75 ± 0,540	3	6,4***
Length of leaves	4,01 ± 0,079	23	4,01 ± 0,155	8	4,75	2[2]	—
Breadth of leaves	5,05 ± 0,086	23	4,73 ± 0,230	8	5,75	2	1,30
Length of leaves / breadth of leaves	0,79 ± 0,0075	23	0,85 ± 0,0123	8	0,82	2	4,2***

[1] The leaves of 4 plants were tattered. [2] A pair of leaves of one of the plants was shrinked.

plant No. 4 conformed in every respect to the normal appearance of the species, while all the others differed, each in its own way. The appearance of the leaves in connection with strikingly weak growth of Nos. 5 and 6 clearly indicated that notwithstanding the chromosome numbers and the comparatively high fertility, these plants must also have been afflicted with hereditary irregularities, probably of a cytological nature.

Two progenies were cultivated from mother plants, the chromosome numbers of which could not be established. Unfortunately, the progeny from *i*41$_5$—44—2 only numbered 3 plants, of which 2 had $2n=36$ and 1 had $2n=37$; the last-mentioned did not present any aberrant external features, and its fertility was good. The offspring from *i*39$_6$—45—2 are reviewed in Table 5. The number of leaf indentations and the leaf measures were computed from the same two pairs of leaves, as referred to above.

The 35-chromosome plants were very clearly distinguishable from those having 36 and 37 chromosomes, by their appearance, while the two latter groups could not be distinguished from each other. The 35-chromosome plants corresponded fairly closely to their mother plant in appearance, and their fertility was also largely comparable to that of the latter. On the other hand, the difference between the 36- and 37-chromosome plants was strikingly small also in this respect. Pure *i*39 was, unfortunately, not cultivated during the same generation; the only material available to be compared with was *i*41, which showed

an average seed-setting in 37 normally fertile plants of 72,1 %. A close correspondence exists between this value and that of the 36-chromosome plants in Table 5; their pollen fertility, too, indicated that the latter plants were fully normal.

Judging from the morphological uniformity and the perfectly good fertility of the 36-chromosome plants, in connection with the numerical superiority of the 35-chromosome plants over those having 36 chromosomes, it seems probable, but by no means certain, that the mother plant had $2n=35$, and that its origin was not preceded by any heterogenetic chromosome pairing. It is true that this leaves the appearance of 37-chromosome plants unexplained, but presuming, on the other hand, that the mother plant had had the constitution $a_1\ a_1\ a_1\ a_2$ instead of $a_1\ a_1\ a_2\ a_2$, some of the 36-chromosome plants should have been partially sterile, and their number should have been greater in comparison with that of the 35-chromosome plants.

Turning now to the offspring from plants with known aneuploid chromosome numbers, the one from $i41a_{21}$—46 is reviewed in Table 6, together with data of pure line plants of the same generation. This offspring, like the one dealt with in Table 5, largely consisted of 35- and 36-chromosome plants; however, the number of hypoploid plants is proportionately much smaller in Table 6 than in Table 5. Contrasting the 35-chromosome plants with the others gives, in a comparison between the two progenies, $\chi^2=4{,}56$ $(0{,}05 > P > 0{,}02)$, indicating that the defects of the mother plants were not identical, even if also the mother plant of the offspring in Table 5 was possibly 35-chromosomic. This is also evident from the characteristics of the progenies, *e. g.*, the difference in pollen fertility of the 35-chromosome plants in the two tables, and the difference between the 36-chromosome groups. The variability within the 36-chromosome group is greater in Table 6 than in Table 5; in the former category, there is also a tendency towards a general deterioration in comparison with the pure line plants in the characteristics examined. The seed-setting varied between 26 % and 67 % and the variation in the shape of leaf and the number of leaf indentations also indicated a disturbed hereditary balance. Probably, all the 36-chromosome plants in Table 5 were of a fully normal type, while those in Table 6 included some which had taken over, to some extent, the hereditary disturbances of the mother plant.

In $i39_{27}$—48, finally, alone among the aberrants observed, it was possible to examine a few PMC's in I M. In 17 of these were found 18 II + 1 I; in almost all the cells the univalent was clearly separated

TABLE 6. *Properties of euploid and aneuploid plants in the offspring of i41a$_{21}$—46 and of normal i41 plants.*

Character	1. $2n = 35$		2. $2n = 36$		3. $2n = 38$		4. pure *i* 41		t-value for the diff. column 1—2	t-value for the diff. column 2—4
	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n	$\bar{x} \pm m$	n		
Seed-setting	44,6 ± 3,57	10	53,2 ± 3,99	12	(31)	1	64,3 ± 2,40	8	1,61	2,38*
Pollen fertility	95,0 ± 0,97	10	97,6 ± 0,43	12	(95)	1	98,3 ± 0,32	8	2,45	1,32
Height of stem	56,2	9	58,1	11	(59)	1	61,6	8	—	—

from the bivalents. In 2 of the cells only 18 bivalents could be recognized, and in 1 cell only appeared a rod-shaped trivalent.

Of the 11 plants in this offspring, which all had 36 chromosomes, 8 were normal, and 3 were aberrant. The latter resembled their mother plant fairly closely, but a full correspondence was found neither between any of the offspring plants and their mother plant, nor between any of the aberrant offspring plants themselves. There were divergencies in shape of leaf, consistence of leaf (degree of stiffness, unevenness of surface, *etc.*), height and breadth of sepals, as well as other characteristics, of which those measured are set out below:

Plant No.	Seed fert.	Pollen fert.	Indentations per leaf
*i*39b 3	2	67	16,75
7	53	95	18,25
10	34	86	19,25
8 sister plants	72,0	98,6	19,07

Attempts to study reductional divisions in the aberrant plants were in vain.

The low frequency of trivalents in $i39_{27}$—48 is surprising, since the frequency of multivalents in both autotetraploid *L. purpureum* and ditto *L. amplexicaule* is as high as about 5,8 per cell (BERNSTRÖM, unpubl.). The sparse occurrence of trivalents may be due to the fact that the supernumerary chromosome, as a result of heterogenetic pairing between partially homologous chromosomes, was of a new, complex type. If the two univalents, which are, in general, likely to be a consequence of a bivalent formed by heterogenetic pairing (see p. 411), move towards the same pole, a plant of the type indicated will originate. The latter may also give rise to up to 5 different aberrant 36-chromosome types in the offspring, apart from gamete selection and the extra variation which may result from crossing-over between the new chromosomes and the original ones. A 37-chromosome plant with an unchanged supernumerary chromosome does not, on the other hand, produce aberrant 36-chromosome types, unless the extra chromosome enters into heterogenetic associations. The argument on p. 411 regarding the cause of pairs of univalents would indicate that the chances of a tendency in this direction on the part of the extra chromosome are quite considerable, but a frequency of aberrant plants of the order of 3 to 8 normal ones rather favours the conclusion that in the case dealt with above the extra chromosome was of a complex

type. This would also fit in with the interpretation of the low frequency of trivalents in the mother plant.

From what has been said above, particularly regarding the offspring from aberrant plants in *L. intermedium*, it is fairly obvious — in spite of the comparatively fragmentary nature of the investigations — that these plants must, in at least the majority of cases, be caused by chromosomal disturbances, and it seems probable that this is always the case. The observations of the meiosis in *L. intermedium* indicate that infrequent but evenly occurring heterogenetic pairing is a characteristic of this species; it is likely that aberrant plants are caused by the same, or a similar, kind of disturbances which are considered, or have previously been considered, to be the cause of fatuoids, speltoids, and compactoids, as explained by WINGE (1924), HUSKINS (1946), and others. The present knowledge of the material does not admit of more exact explanations than those presented above; they show fairly clearly, however, that aberrant plants may be caused both by aneuploid chromosome numbers and, even more, by different chromosome-structural recombinations. Each of the plants Nos. 3, 4, 5, 7, and 10, at least, probably represents one type of irregularity, and the differences in appearance and degree of fertility reduction between these and the others strongly favour the presumption that the number of types found is even higher.

On the other hand, the full fertility and the normal appearance of several 37-chromosome plants in different offspring show that it is not possible to pick out all aneuploid plants except by determination of the chromosome numbers, and the real number of aberrant plants in the material cultivated may therefore, in fact, have exceeded that which was actually found. As a matter of fact, this may possibly apply to *L. hybridum* as well.

V. DISCUSSION.

Although the investigations of the 4 species dealt with above were carried out rather as an orientation and an appendix to the attempts to establish the mutual relationships between the species and were, therefore, fairly fragmentary, they have, nevertheless, presented a general picture of the cytogenetic structure of the species which was the purpose of the investigation. It was found that all the 4 species are strictly self-pollinating and, consequently, at least as a rule consist of pure lines. As far as the cytological and genetic relations between the lines

of one and the same species are concerned, the species may, however, be divided into 3 different types. The first of these, represented by *L. amplexicaule*, is distinguished by the fact that there are both genetic and chromosome-structural divergencies between the lines (*cf.* BERNSTRÖM, 1952). The second group includes *L. purpureum* and *L. hybridum*, in which the genetic differences between the lines are obvious from their morphological and physiological divergencies, but in which no chromosomal divergencies could be traced. The third group, finally, is represented by *L. intermedium*, in which hardly any genetic, and even less any chromosomal divergencies were found. The supposed, or known, reasons for these differences have already been briefly dealt with above, but it may not be out of place to review them summarily.

1. L. AMPLEXICAULE.

There is no given explanation of the unique position of *L. amplexicaule* by virtue of the general appearance of more or less pronounced chromosome-structural differences between the lines. There are several possible reasons. This species may be much older than *L. purpureum* with which it should, in the first place, be compared, and this may have led to the concentration of spontaneous chromosome-structural recombinations to a far higher degree in *L. amplexicaule* than in *L. purpureum*, and may, in at least the majority of lines, have resulted in each line acquiring a characteristic chromosomal structure of its own. Any presumption of this nature must, however, remain pure guess work in the absence of more detailed knowledge regarding the age and origin of the two species. Similarly, it is only by way of conjecture that the assumption may be put forward that *L. amplexicaule* might, cytologically, be less stable than *L. purpureum* instead of being older than the latter. An assumption in this direction seems, however, most improbable.

As already pointed out, however, *L. amplexicaule* is evidently characterized by a far greater variability in South-Eastern Europe and Western Asia than in any other part of its distribution area, while at the same time a number of species which, judging by appearance, should be closely related to *L. amplexicaule*, are also found in part of these areas. A number of differing types of *L. amplexicaule* have thus been described from Greece, Asia Minor, and the Eastern Mediterranean as well as from some neighbouring areas (for instance, *L. amplexicaule* f. *incisum*, f. *curdicum*, f. *tel-avivensis*, f. *aleppicum*, var.

graecum, var. *orientale*, var. *Nemetzii*), while distinct types from other geographical areas have been reported only occasionally. The systematic value of some of the varieties mentioned above may be uncertain, but the number of distinguishing names of varieties from these areas which are but little studied botanically, confirms in fact the existence of the wide variation within the species, which is indicated by the cultivated material as well as by studies on herbarium material. Among other species within the genus, belonging to its annual group, are *L. aleppicum*, *L. macrodon*, *L. eriocephalum*, *L. bifidum*, *L. Ehrenbergii*, and *L. rumelicum* in the Eastern and North-Eastern Mediterranean, and several of these indicate, by their appearance, a closer relationship to *L. amplexicaule* than to *L. purpureum*. The recent discovery of what is probably a new species among some samples of *L. amplexicaule* (in a wider sense) and *L. purpureum* from Turkey, shows, incidentally, that unknown species of the genus may still exist in these areas; the supposedly new species will be described elsewhere by Dr. BURTT, of the Botanical Gardens, Kew, who has been kind enough to assist in the identification of several of the Mediterranean *Lamium* species which were included in the cultivated material.

L. aleppicum, *L. Ehrenbergii*, *L. bifidum* subsp. *balcanicum*, and the supposedly unknown species referred to above (labelled *No. 82*), have been cultivated for a few generations; they did not, however, thrive well and therefore produced chasmogamic flowers only very sparingly, as a result of which only a few cross-pollinations could be made between them and *L. amplexicaule*, and no hybrids have so far been obtained. In *L. Ehrenbergii*, *L. bifidum* subsp. *balcanicum* and *No. 82*, the chromosome number was found to be $2n=18$, while *L. aleppicum* has $2n=16$. Hybrids both between *L. purpureum* and *L. bifidum* subsp. *balcanicum* and between *L. purpureum* and *No. 82* are quite sterile. At first metaphase the latter form 0—3(—5) bivalents; the reductional division in the former has not been studied.

Only the different chromosome numbers of *L. amplexicaule* and *L. aleppicum* together with the cytological disturbances in hybrids between *L. amplexicaule* and *a*33, discussed previously, as well as the pronounced sterility in the hybrids between *L. amplexicaule* and *a*81, point more or less directly to the existence of cytological divergencies between *L. amplexicaule* and other annual species and varieties of the genus in the Eastern Mediterranean. In addition to this, the cytological differences between the genomes of *L. amplexicaule* and *L. purpureum* have been referred to earlier. Thus, there is but scant support for the

assumption that the cytological differentiation between the lines of *L. amplexicaule* may have been caused by hybridization with one or more species or varieties, chromosome-structurally different from *L. amplexicaule*. Yet, it seems likely to presume that the chromosome-structural differences between the lines may have been caused in this way, this presumption being based not only upon such direct observations as have been possible, but also, and in the first place, on the fact that more effective means in nature for the production of such differences are, so far, unknown.

MÜNTZING (1938), in his analysis of the partial sterility in *Galeopsis Tetrahit*, deals with the conditions necessary for the origin of chromosome-structural divergencies leading to intraspecific partial sterility, and discusses its various cytological aspects. The subject has since been further treated by many others. In their surveys of the research in this field, STEBBINS (1950), HEISER (1949) and others show what a common phenomenon hybridization between species probably is, with a resulting formation of new varieties and chromosome-structural recombinations. In the present investigation, examinations of meiosis in hybrids between lines of *L. amplexicaule* as well as in hybrids between these lines and *L. hybridum*, indicate that chromosome-structural divergencies between the lines of *L. amplexicaule* are probably more extensive than would have been supposed on the sole evidence of meiosis and the fertility of their hybrids. This lends support to the assumption that the differentiation of the present lines has been proceeded by hybridization with structurally diverging varieties or species. The common existence of structural divergencies between the lines and the probable extent of these divergencies indicate that cross-pollination has probably not been all too rare.

If the problem is regarded in this way, the question arises as to how this hybridization could take place in a strict self-fertilizer like *L. amplexicaule*. Experiences from the study of this species as well as the other ones show, however, that a certain, although probably very slight, amount of cross-pollination may in fact take place between lines of *L. amplexicaule* and also between this and other species. As pointed out earlier (BERNSTRÖM, 1952), about 10 % of the chasmogamic flowers in *L. amplexicaule* fail to self-pollinate, and, further, a real case of vicinism was observed. Even taking into consideration the fact that the material was cultivated in greenhouses, which may very well have impaired the ability of the flowers to self-pollinate, the said percentage suggests that in nature some flowers do, in fact, fail to self-

pollinate and thereby expose themselves to cross-pollination, primarily through the activity of insects.

Attention has also been drawn previously (p. 406) to the nature of *L. intermedium* as an allotetraploid between *L. purpureum* and *L. amplexicaule*, and to the fact that this is surprising in view of the impossibility, despite repeated attempts, to hybridize the two species. The existence of *L. intermedium* shows that cross-pollination must, as a matter of fact, occur to some extent between its parent species even if, regarded as an individual incident, it is probably very rare. At least in Northern and Central Europe, both species are very common as weeds, growing often intermingled and in great abundance. When stating that *L. intermedium* is a hybrid, nothing is of course known as to which of the parent species functioned, or generally functions, as mother, but probably pollination takes place in both directions.

It is, then, reasonable to expect that cross-pollination should sometimes occur also between pure lines of *L. amplexicaule* in nature, and presumably also with other species than *L. purpureum*. It should also be noted in this connection that it is not quite certain that this species, in what is probably its variation centre in South-Eastern Europe and Western Asia, is as strictly autogamous as in Northern Europe. According to RICK (1950), male-sterile lines of *Lycopersicum esculentum* from California get their flower morphology changed when cultivated in the area of origin in Peru, the degree of cross-pollination being also considerably higher there than in California, particularly through the activity of insects. He also refers to HARLAND's (1948) interesting experience that *Pisum sativum* is subjected to a pronounced degree of cross-pollination when cultivated in Peru instead of in Europe, or in Abyssinia, which is believed to be its area of origin. In these parts vicinism is rare, while 3 % of the plants in Peru are hybrids, originated through the activity of a carpenter bee. Examples like these prove that it is not certain that a species should present the same kind of flower biology throughout its distribution area.

Thus, in view of what has been stated above it is not improbable that the chromosome-structural divergencies between the lines of *L. amplexicaule* have originated through hybridization between closely related though chromosome-structurally diverging species within the area of origin of *L. amplexicaule*, or when following human culture from this area. Further, it is most probable that hybridization between the lines of this species occurred sparingly but regularly, during the continued spreading with human culture, which resulted in this spec-

ies becoming a very common weed in the Old World and also in parts of other continents. Then, this has resulted in the origin of new lines through recombination of the parental genes and the survival of the types best suited to the existing external conditions; and if the parent lines differed in their chromosomal structure, the new lines may, through recombinations of the latter, have acquired new chromosomal types.

No clear correlation does exist between the degree of fertility reduction in the F_1 hybrids and the distance between the places of origin of the parent lines. Hybrids between lines from, *e. g.*, the most distant places, in Sweden and Syria, belong to the most sterile hybrid combinations obtained, as well as to the most fertile ones. The lack of correlation indicates that it is not any special race of the species with a chromosomal constitution of its own, which has spread over Europe, but rather that the chromosomal constitution of those lines which have extended the distribution of the species towards the North-West, and of the new lines which may be assumed to have arisen by occasional interlinear hybridization during the spreading of the species, has been decided by chance.

There is, so far, no reason for dividing the species into any smaller units, from a biosystematic point of view, on the basis of the partial sterility which characterizes, to a different degree, the hybrids between the lines of the typical *L. amplexicaule* (*cf.* CLAUSEN, KECK, and HIESEY, 1945). It is impossible to regard *L. amplexicaule* as anything but a single systematic unit, so long as the lines cannot be divided into groups between which the hybrids are, throughout, characterized by a strongly reduced fertility, but within which the fertility is good; and, as pointed out before (BERNSTRÖM, 1952), it is highly probable that the greater the number of lines included in the investigation, the more difficult will it be to divide them into such groups. Out of the material investigated only *a*33 may be clearly classified as a type of its own, differing throughout from the others by reason of considerably reduced fertility and more extensive chromosome-structural divergencies in the hybrids than found in hybrids between other lines. To this should be added the morphological type of the line, which differs from that typical of *L. amplexicaule*.

Using the biosystematic classification, the lines investigated must, with the exception of *a*33, be regarded as belonging to one and the same ecospecies of *L. amplexicaule*, the great majority of lines being probably referable to a common ecotype, while *a*83 should, as pointed out

earlier (BERNSTRÖM, 1952), be considered as belonging to another ecotype. *a*33 may be looked upon as representing another ecospecies within *L. amplexicaule*, the species taken in a wider sense. A more representative experimental material from those areas where the variation of the species and the genus is greatest, would probably have provided that picture of the cytogenetic structure of *L. amplexicaule* of which we have, evidently, so far only been able to catch a glimpse, but which, judging from our present knowledge, should be richly faceted.

It has been pointed out earlier (BERNSTRÖM, 1952) that the pollen fertility of the interlinear hybrids within *L. amplexicaule* is more commonly reduced, and to a higher degree, than was found to be the case in an analogous investigation of hybrids between 12 lines of *Galeopsis Tetrahit* (MÜNTZING, 1938); *i. a.*, the pollen fertility of three of the hybrids within pure *L. amplexicaule* is less than 50 %, while this is not the case with any of the hybrids in *Galeopsis Tetrahit*. No clear correlation has been found, in any of the species, between the degree of sterility and the extent of the meiotical irregularities which very often characterize the partially sterile hybrids, in spite of the fact that the sterility is, quite predominantly, very likely caused by chromosomal aberrations. Therefore, it is very hazardous to make comparisons between the species as regards the frequencies of sterility-producing factors by estimating the degree of meiotical disturbances; and the more so as the one species is diploid but the other tetraploid.

The chromosome number must be of great importance for the effect of sterility-producing factors. It is a well-known fact that, on the whole, tetraploids are less sensitive than diploids to deficiencies, duplications and other kinds of chromosomal aberrations, as well as to the size of the latter. Regarding the influence of chromosomal aberrations upon fertility, LAMM (1945) and others have pointed out that these must have a less deleterious effect in autotetraploids than in diploids, the greater number of possible recombinations of structural differences, *etc.*, rendering those combinations fewer which are lethal to the gametes. This applies also to the case where several different sterility-producing factors are involved. The difference between diploids and tetraploids must be most pronounced when the latter are autotetraploids, and less so for allotetraploids, the genomes of these having only part of the genes in common. In allotetraploids, the harmful effect of chromosomal aberrations is not suppressed by a multiplication of the possible recombinations, but instead by the partial genetic homology between the genomes which will always exist.

According to the terminology of STEBBINS (1947), *Galeopsis Tetrahit* may be regarded as an intermediate case between segmental and true allotetraploidy. The predominant formation of 8 bivalents in the F_1 hybrids between *G. pubescens* and *G. speciosa* indicates segmental allotetraploidy, as does the existence of polymeric genes in *G. Tetrahit*, while the scarce occurrence of multivalents points to true allotetraploidy (MÜNTZING, 1930, 1932 a, 1938). The common occurrence of univalents may, however, be due to heterogenetic association. Therefore, there is reason to suspect the existence of extensive homology between the genomes of *G. Tetrahit*, which must have a counteracting effect on the manifestations of sterility. Provided that the frequencies of chromosomal aberrations are about the same in interlinear hybrids of a diploid and a more or less allotetraploid species, there is, consequently, reason to expect that, in spite of a quite predominant bivalent formation in the latter, there will be found a difference in the degree of fertility of the hybrids, reminiscent of that between *L. amplexicaule* and *G. Tetrahit*.

It has been mentioned above that partial sterility of F_1 hybrids within *L. amplexicaule* is removed by doubling the chromosome number (BERNSTRÖM, 1952). The reason of this may at least in part be the greater recombination possibilities of autotetraploids as compared with those of diploids, as discussed above; the slight partial sterility which must still have remained, may have been too inconsiderable to be clearly perceptible, or may have been hidden by the marked increase of fertility which characterized some of the hybrids in comparison with the pure tetraploid lines.

It is also possible, that the perfectly good fertility of the hybrids, as compared with that of the parent lines, can be explained by preferential, *i. e.* intralinear, pairing of the sterility-producing chromosomes. Accordingly, the chromosomes should, at least to some extent, form bivalents instead of quadrivalents. It does not seem probable, however, that preferential pairing contributes towards removing sterility in the tetraploid hybrids to any appreciable degree. Arguments against this are the rather high frequency of multivalents (*viz.* about 5,8 per cell) in I M of autotetraploid *L. amplexicaule*, as established by preliminary investigations, as well as the rather inconsiderable sterility which characterizes three of the corresponding diploid hybrids, the bivalent formation in a couple of these being also almost regular. It seems more probable that the chromosome pairing in the tetraploid hybrids is

inter- as well as intralinear, and that the removal of the partial sterility depends exclusively on the genetic conditions of the autotetraploids.

2. OTHER SPECIES INVESTIGATED.

Of these, *L. purpureum* proved, with regard to its comparatively rich variation, to resemble *L. amplexicaule* most closely; like the latter it is, *i. a.*, probably differentiated in various ecotypes (see above, p. 382). On the other hand, the somewhat greater uniformity of *L. hybridum* and even more that of *L. intermedium*, is obviously a consequence of these two species being allotetraploids. Systematically, *L. purpureum* appears to hold a somewhat isolated position in the genus. It has sometimes been grouped in another section of the genus than that in which *L. amplexicaule* and related species have been placed (BOISSIER, 1879). In the present investigation, no cytological divergencies of the kind which characterize *L. amplexicaule* have been found in *L. purpureum* to indicate interspecific hybridization, as a means of extending the variability within the species. The lines investigated are so many and so different, and originate from such extensive parts of the distribution area, that there are grounds to believe that, cytologically, the species is at least for the most part homogeneous.

Turning to the origin of *L. purpureum*, it may be presumed that this species, as it now appears, reached its present variation from a fairly uniform original type through mutations which, together with a sporadic interlinear hybridization of the same kind as that supposed in the case of *L. amplexicaule*, have brought about a successive differentiation of the species. Such a differentiation might have been facilitated and accelerated while following the human culture to the westward and northward from the centre of origin which, according to HEGI (1927), has probably been in Western Asia. It is, however, also possible that the present *L. purpureum* originates from a partly allogamous and already varied species. Autogamous pollination may, under the influence of cultivation, have been favoured in the manner supposed by STEBBINS (1950) to be characteristic of annual species. We have no knowledge of the type of pollination of this species in, for instance, the Eastern Mediterranean countries, but on the evidence of what has been said in this respect about *L. amplexicaule*, it is by no means out of the question that the degree of self-pollination in Northern Europe differs from that of, *e. g.*, the Mediterranean area.

It would seem that a concrete example of a hereditary differentiation of the species according to its distribution is to be found in

the distribution mentioned above (p. 382) of summer-annual lines in Northern and Central Europe as against winter-annuals or/and short-day-flowering lines in Central and Southern Europe and the Mediterranean area.

The cytological uniformity of *L. purpureum* is, however, an indication against partial allogamy in the original type. As a rule, cross-pollinating species may be expected to be characterized by more or less pronounced structural heterozygosity (MÜNTZING, 1939). If *L. purpureum* has developed from allogamy to autogamy, cytological differences could be expected to have been stabilized between the differentiated pure lines, the differences manifesting themselves in partial sterility of the interlinear hybrids.

If, therefore, the origin of the present *L. purpureum* is fairly unknown, we are in a somewhat better position in the case of *L. hybridum* and in a very much better position in that of *L. intermedium*. As pointed out earlier, *L. hybridum* is an allotetraploid, the parent species of which it has been impossible to identify experimentally, but of which *L. purpureum* is probably one; this question will be dealt with more fully in a later paper. The absence of interlinear partial sterility in *L. hybridum*, although represented in the experiments by lines of quite different appearance and from entirely different parts of the distribution area of the species, raises, on the whole, two alternatives with reference to the question of the origin of this species. One is that *L. hybridum* originated once only and, if so, very long ago, since the species now presents pretty different lines. Since *L. amplexicaule* is, as far as is known, the only species which is compatible with *L. hybridum*, and since their hybrids are entirely sterile, the variation in *L. hybridum* has not, in all probability, been accelerated through interspecific hybridization; moreover, the cytological homogeneity of the species is most strongly contradictory to such a presumption. Instead, the variation within the species must be ascribed to mutations in connection with recombinations caused by occasional interlinear hybridization, in the same way as with the diploid species.

The alternative explanation of the origin and, at the same time, of the cytological homogeneity of *L. hybridum* is based on the possibility of repeated originations of the species, each of which resulted in exactly the same cytological structure. In practice, this would presuppose both parent species to be cytologically homogeneous and their genomes to be inhomologous, so that there would be no, or only very rare, pairing between them. *L. hybridum* may have originated through somatic as

well as through gametic doubling of the chromosome number; in the latter case any pairing between the chromosomes would hardly have taken place. Another prerequisite would be that the two parental genomes were fairly ideally suited to each other genetically so that the resulting new *L. hybridum* would possess good vitality and fertility right from the beginning. In most cases newly formed allopolyploids will probably achieve such a condition only after several generations of recombination through heterogenetic pairing, but there are examples of an almost direct synthesis of a fully fertile, vital allopolyploid, *e. g.*, *Madia nutrammii* (see CLAUSEN, KECK, and HIESEY, 1945).

Considering the fairly remarkable variation between the lines of *L. hybridum*, one may be inclined to favour the latter alternative. As stated above, *L. purpureum* is probably one of the parent species of *L. hybridum*, on cytological as well as on systematical grounds. Its cytological uniformity might contribute towards the latter of the two alternatives; on the other hand, however, this is contradicted by the fact that the chromosome pairing in hybrids between *L. hybridum* and tetraploid *L. purpureum* clearly suggests that chromosome-structural recombinations have occurred in *L. hybridum*, provided that *L. purpureum* is one of the parent species (BERNSTRÖM, unpubl.). The question can only be elucidated by tracing the two parent species through an artificial synthesis of *L. hybridum*; for the present, however, the circumstance mentioned must be considered to be the most important argument, *L. hybridum* thus having probably originated only once and much longer ago than *L. intermedium* (*cf.* below).

It may be added that systematical and, to some extent, plant geographical reasons support the suspicion that *L. bifidum* subsp. *balcanicum* is the other parent species of *L. hybridum*. In the material cultivated, one line of this species was found to be short-day-flowering. It is compatible with *L. purpureum*, and the F_1 hybrids are long-day-flowering like the latter, and like *L. hybridum*. Further, the hybrids are completely sterile. Their meiosis has not yet been studied but hybrids having the double chromosome number have already been produced, and attempts are in progress with a view to using these in testing the origin of *L. hybridum*.

With reference, finally, to *L. intermedium*, it has already been pointed out that this species is in fact an allotetraploid between *L. purpureum* and *L. amplexicaule*, and circumstances have been brought forward which strongly favour the idea that *L. intermedium* originated once only, and that this occurred fairly late, from a biological point of

view (*cf.* p. 406). Among the indications of a comparatively low age of the species is also the cytological instability which, on one hand, appears during meiosis by the occurrence of univalents as well as multivalents, and, on the other, gives rise to the segregation of aberrant plants. It seems probable, however, that it will take a longer time for this species to achieve greater cytological stability through selection of, in the first place, chromosome-structural changes, than is required for chromosomal and genetic mutations of different kinds to differentiate the evidently very homogeneous species into various pure lines distinguishable morphologically as well as physiologically. The fertility of *L. intermedium* is good, in spite of the meiotical irregularities, and its wide distribution indicates a good vitality and competitive ability; and the improvement of these qualities may be presumed to be but very slowly effected by selection of chromosome-structural changes, resulting in a greater cytological stability. It would probably be incorrect, therefore, to attach the same importance to the defective cytological stability of *L. intermedium* as to its morphological and physiological uniformity, as an indication of the low age of this species.

Obviously, in a species so cytologically unstable as *L. intermedium*, new types with changed chromosomal structure may be expected to originate. Even if they are perhaps not always characterized by external features, one may expect to identify them by reduced pollen fertility in their hybrids with other lines. The material investigated, 7 lines, is too small to form a basis for any definite statements regarding the existence of this kind of »structural» lines, and more extensive investigation in this direction would have been of interest for the insight it might have provided into the differentiation process of an at least comparatively recent species. The present investigation does, however, indicate that a differentiation of the expected nature would not appear to be any common phenomenon in *L. intermedium*.

SUMMARY.

(Has reference also to Part I.)

(1) As part of an investigation on species hybrids regarding the relationships between the North European annual *Lamium* species, preliminary cytogenetic studies on the species concerned were also carried out. The purpose of these studies was partly to facilitate the investigation of the relationships between the species (a treatise on this subject will be published later) by increasing our knowledge of the

species themselves, and partly to throw light upon more general questions regarding the properties of diploid as well as allotetraploid autogamous species.

(2) The species investigated are *L. purpureum*, *L. amplexicaule*, *L. hybridum*, and *L. intermedium*. The two former are diploid ($2n=18$), while the two latter are tetraploid ($2n=36$). Between 7 and 16 pure lines of each species were studied.

(3) All the species are highly autogamous. The percentage of good pollen is always very high in the lines of all species. The average for each of the diploid ones is about 99 %, and that of each of the tetraploids nearly 98 %. This slight difference is statistically quite significant, and is attributed to the tetraploid condition in itself, rather than to visible disturbances in meiosis (p. 412). The seed-setting, too, is lower in the tetraploids, although it is good in all species.

(4) Almost all F_1 hybrids investigated between 16 pure lines of *L. amplexicaule* were characterized by partial sterility. The reduction in fertility, measured on the percentage of good pollen, varied from a hardly noticeable deterioration, down to about 30 %. Each hybrid combination was characterized by a certain definite degree of sterility. Differences as to the degree of fertility reduction in their hybrids probably exist between the lines. The majority of the lines, and probably all of them, each represent one constitutional type, as far as factors producing sterility are concerned.

(5) The seed fertility deteriorates in about the same degree as the pollen fertility in intraspecific hybrids of *L. amplexicaule* (the correlation coefficient $= +0{,}88$). It is demonstrated, however, that in the more sterile hybrid combinations the fertility reduction is smaller as regards the seed-setting than regarding the pollen fertility, while with a slightly reduced fertility the case is rather the reverse. This is contrary to the conditions in other partially sterile materials of different kinds within *Lamium*, *e. g.* artificial polyploids, aneuploids, and species hybrids, in which the seed fertility is always inferior to the pollen fertility. The explanation is supposed essentially to be found in different degrees of genetic diversity between the parents, or — regarding the polyploids, *etc.* — in the degree of their own genetic unbalance.

(6) Some hybrid combinations within *L. amplexicaule*, which are partially sterile, are characterized, as tetraploid, by a seed-setting which is as good as, or superior to, that of their tetraploid parent lines. Therefore, the sterility in the diploid hybrids cannot be genic. The reason

of the removal, in the tetraploids, of the partial sterility is supposed to be found in the genetic conditions of autotetraploids (p. 428).

(7) Meiotic disturbances, especially in the form of univalents and inversion bridges, are common in hybrids within *L. amplexicaule*. No correlation has been found betweeen the frequency of disturbances and the degree of sterility. However, the partial sterility is probably caused by predominantly small, chromosome-structural divergencies between the lines.

(8) The hybrids between a line of *L. hybridum*, which is an allotetraploid, and 3 different lines of *L. amplexicaule*, are characterized by far greater differences between themselves in the pairing conditions at I M, than are the hybrids between the *L. amplexicaule* lines. The explanation of this is probably that the chromosome-structural divergencies between the lines are more extensive than would appear from the chromosome pairing in the hybrids, and that these divergencies may be brought out more clearly by pairing with a genome which is only partially homologous than by pairing with the genome of the same species.

(9) Judging from several characteristics, interlinear hybrids in *L. amplexicaule* and *L. purpureum* were found to be generally characterized by heterosis, or tendencies towards heterosis. On the other hand, this is not the case with *L. hybridum* and *L. intermedium*. Attention is drawn to instances of genic differences between the lines of *L. purpureum*, which give rise to sterile dwarfs in intraspecific F_2 generations or cause dwarfishness in F_1 hybrids with *L. hybridum*; they are discussed as possible contributory causes of heterosis. There is no correlation, in *L. amplexicaule*, between the degree of heterosis and the degree of partial sterility.

(10) Neither in *L. purpureum*, nor in *L. hybridum*, nor yet in *L. intermedium* could interlinear partial sterility be established in F_1 hybrids, the lines in these species appearing to be structurally identical, in contrast to those of *L. amplexicaule*.

(11) I M in *L. hybridum* is highly regular. Spontaneous aneuploids are probably rare; only one plant with $2n=37$ was found. Plants with chromosome numbers varying between 33 and 38 were artificially obtained.

(12) *L. intermedium*, which is an allotetraploid between *L. purpureum* and *L. amplexicaule*, is a very homogeneous species, hardly any differences between the lines cultivated being traced. Univalents are common in I M, and multivalents occur; the irregularities are pre-

sumed to result from heterogenetic pairing. In consequence, about 2 % of the plants are characterized by chromosomal aberrations of various kinds, and are generally distinguished by divergent appearance and reduced fertility. 10 aberrant plants are described, representing at least 5 and probably more kinds of cytological deviations. The offspring from some of the plants are described. Various arguments are put forward in support of the assumption that *L. intermedium* originated comparatively recently, and on one occasion only.

(13) The possible development of the species investigated is discussed, particular attention being devoted to the appearance of partial sterility in *L. amplexicaule*. This is probably caused by hybridization with chromosomally divergent, but closely related species, which are found in Western Asia. The structural divergencies between the lines may since have been developed and spread within the species by occasional interlinear hybridization.

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DNA SYNTHESIS IN THE MICROSPOROCYTES OF LILIUM HENRYI

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SEVERAL recently published studies have been concerned with the question of desoxyribose nucleic acid (DNA) in the meiotic nuclei of plants (BRYAN, 1951; OGUR *et al.*, 1951; SCHRADER and LEUCHTENBERGER, 1949; SPARROW *et al.*, 1952; SWIFT, 1950; TAYLOR, 1953). The data and conclusions, briefly reviewed below, show considerable disagreement; they are, in fact, not strictly comparable since the materials as well as the methods of analysis employed are quite diverse.

The labelling of newly formed DNA molecules with P^{32} and their subsequent detection by autoradiography has furnished a considerable amount of information on mitotic chromosome metabolism (HOWARD and PELC, 1951 a, b). This method differs from those based on histophotometric or microchemical analyses in that it does not reveal the amount of existing DNA but only the formation of new DNA in which P^{32} has been incorporated in a form not removed by hot HCl extraction. I have used P^{32} autoradiography in a preliminary investigation of the pattern of DNA synthesis in the microsporocytes of *Lilium Henryi*. This investigation is similar to the study of TAYLOR (1953) on *Lilium longiflorum* and *Tradescantia paludosa*.

P^{32} was administered by placing cut inflorescences bearing buds of varying length, with correlated developmental stages (*cf.* ERICKSON, 1948; GREGORY, 1940) in White's medium to which the isotope had been added. The final P^{32} concentrations ranged from one to four microcuries per ml. of solution. The inflorescences remained in this medium from 24 to 250 hours, during which time both the growth of the buds and the overall uptake of solution were recorded. The anthers were then dissected out, measured to the nearest 0,1 mm, fixed in acetic alcohol, and processed into Feulgen-stained section and unstained squash autoradiograph slides by a stripping film technique (DONIACH and PELC, 1950). A 12 minute N HCl hydrolysis at 60° C was used to remove the non-DNA phosphorus. (A DNA-ase enzyme experiment was carried out to test the assumption that the remaining P^{32} was indeed localized in the DNA. The results showed this assumption to be essent-

ially correct.) Examination of the autoradiographs derived from the anthers of 50 flower buds, distributed over 20 individual plants, led to the following conclusions:

(1) New DNA is synthesized in the microsporocyte nuclei at some time during the interphase following the last pre-meiotic mitosis and preceding leptotene.

(2) This period of synthesis is not coextensive with the interphase but ends before the leptotene chromosome strands can be seen.

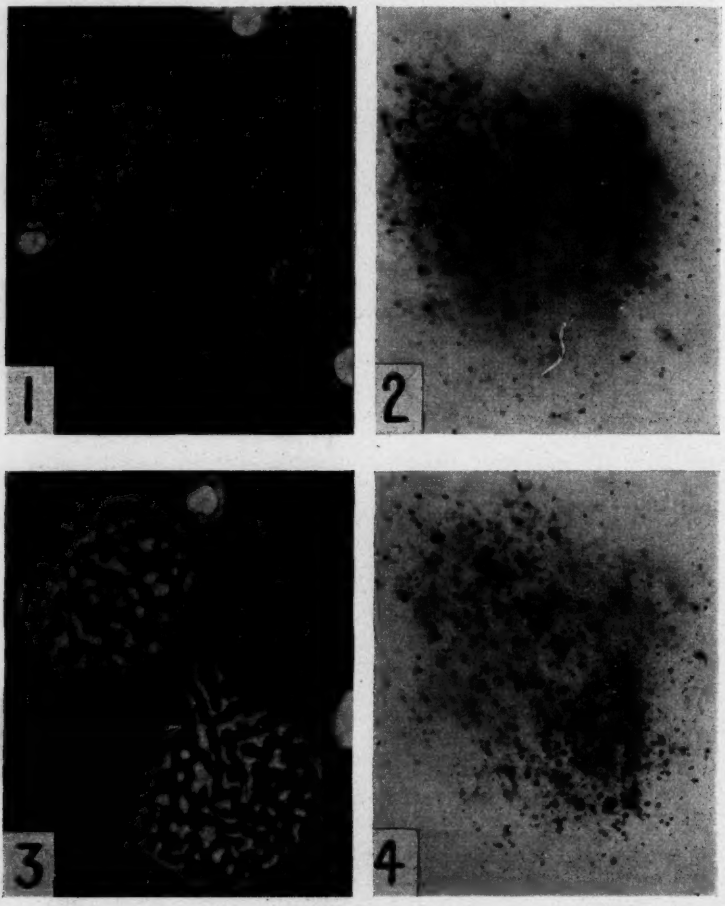
(3) There is no evidence for the formation of new DNA (*i. e.* incorporation of P^{32}) in microsporocyte nuclei between leptotene and the stage of uninucleate microspores with partially completed exines. Later stages were not examined.

(4) It is possible that either the rate of DNA synthesis or the total amount of newly synthesized DNA is less in the microsporocyte nuclei than in tapetal or parietal nuclei. One of these assumptions could account for the apparent difference in grain density usually observed over these three types of nuclei.

(5) The proportion of nuclei synthesizing DNA in the tapetal tissue layer varies with the developmental stage of the anther. Few or no tapetal cells showed autoradiographs if P^{32} was available when the microsporocyte nuclei were in the later portion of the pre-leptotene interphase or in leptotene; almost all showed evidence of DNA synthesis during mid-zygotene to early pachytene. The tapetal cells become multinucleate during the latter period.

(6) Some synthesizing nuclei were found in the parietal tissue during all but the latest stages of anther development studied.

The method of analysis employed in arriving at these conclusions is illustrated in Fig. 5, where the results obtained in the buds of three of the 20 plants studied are graphically represented. Since the shortest period of P^{32} administration was about 24 hours, the problem of distinguishing between pre-leptotene meiotic and mitotic synthesis was introduced. On the assumption that the last pre-meiotic mitosis includes the usual period of DNA synthesis in the preceding interphase, any synthetic activity following this period can be considered meiotic. Taking into account the uncertainty involved in determining the cytological stage of the microsporocytes at the beginning of the treatment, I used the estimated anther length at the last mitotic telophase as the critical length: autoradiographs over sporogenous nuclei were not considered as evidence for pre-leptotene meiotic DNA synthesis if the estimated anther length at the beginning of P^{32} treatment fell short of



Figs. 1—4. Photomicrographs of unstained squash autoradiograph preparations. — Fig. 1. A tapetal and a pre-leptotene sporogenous nucleus as seen with a phase microscope focused through the film on the tissue. — Fig. 2. The same field as seen with the phase plate removed and the objective focused on the film: an autoradiograph is evident over the sporogenous nucleus, indicating P^{32} incorporation into DNA, none over the tapetal nucleus. (40 hours of exposure to active solution.) — Figs. 3 and 4. Two tapetal nuclei at a later stage of anther development and their autoradiographs. (133 hours of exposure to active solution.) The difference in the number of grains visible in Figs. 2 and 4 is due primarily to photomicrographic focusing difficulties and not indicative of corresponding differences in P^{32} present in the nuclei. (Such comparisons are meaningful only when made on the different nuclei of a single anther.)

that length by more than about 12 hours' growth, *i. e.* approximately 0,3 mm. A supplementary method of distinguishing between meiotic and mitotic DNA synthesis, applied in border-line cases, is based on the absence of synchronization in the sporogenous cells during their last mitotic divisions. In the course of 24 hours of P^{32} availability only a portion of the nuclei should be labelled if this labelling is the result of mitotic DNA synthesis. Samples of autoradiographs over tapetal and sporogenous nuclei are shown in Figs. 1—4.

OGUR *et al.* (1951) analysed the DNA content of *Lilium longiflorum* microsporocytes by a microchemical procedure (OGUR and ROSEN, 1950) and reported a gradual increase in DNA content beginning at diakinesis and ending in a doubling just prior to microspore mitosis, followed by a further gradual increase until anthesis. They give no data on earlier stages. Applying a different method of chemical analysis to the corresponding cells of *Trillium*, SPARROW *et al.* (1952) showed that the DNA content increased in the microsporocytes during pachytene and diplotene and then remained constant until the end of the second meiotic division. Their data differ from those of OGUR and his group in this latter constancy. Unfortunately, neither the pre-pachytene microsporocytes nor the microspores after the early post-telophase II interphase were included in the *Trillium* analysis. SWIFT (1950) used histophotometric methods (Feulgen) on *Tradescantia* and found a DNA increase from the diploid to the tetraploid amount in the leptotene and possibly the zygotene stage of the microsporocytes, but no further increase until late microspore interphase. The period of increase here ends earlier than in the *Trillium* nuclei analysed by SPARROW. The findings of SCHRADER and LEUCHTENBERGER (1949), also based on Feulgen photometry in *Tradescantia*, agree with those of SWIFT in pointing to a DNA content in pachytene very much higher than that of any diploid somatic nucleus, no further increase between that stage and post-telophase II interphase, and an approximate 1 : 4 relationship in the DNA content of microspore interphase and pachytene nuclei. SCHRADER and LEUCHTENBERGER present no details of the pre-pachytene period of meiosis. BRYAN's (1951) histophotometric data on *Tradescantia* microsporogenesis are in fair agreement with the extraction analysis data of OGUR *et al.* in *Lilium*. He finds a progressive increase in DNA content from the time of tetrad formation (telophase II) to microspore mitosis, but gives no data bearing on meiosis proper. Most recently, TAYLOR (1953) has investigated the problem in meiosis of *Lilium longiflorum* and *Tradescantia paludosa* by means of P^{32} auto-

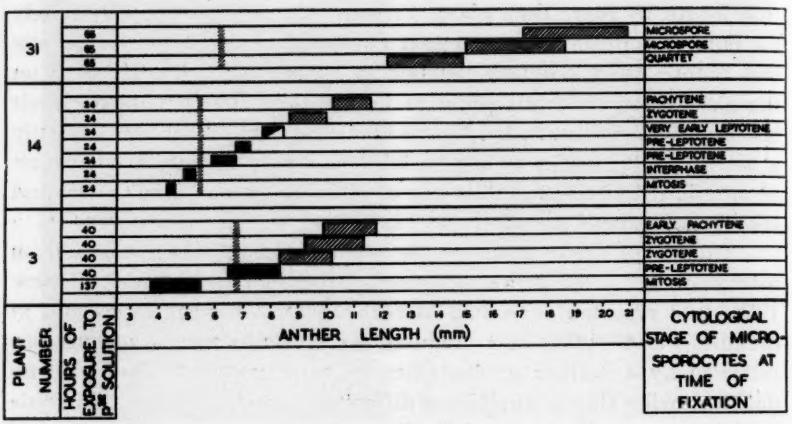


Fig. 5. Diagram representing anther growth in 15 buds of three inflorescences during exposure to P^{32} for varying periods. The growth, indicated by the horizontal extent of the blocks, is recorded in millimeters of anther length at beginning and end of treatment. (Initial anther length is assumed to be equal to measured initial bud length × measured final anther length/measured final bud length.) Solid blocks indicate the presence of autoradiographs over the sporogenous nuclei, cross-hatched blocks their absence, and the half-solid block the presence of autoradiograps of lower than usual grain density. The cytological stage of the microsporocytes at time of fixation is indicated. (The designation »pre-leptotene» denotes interphase nuclei which are distinctively pre-meiotic by virtue of their large, granular appearance.) The vertical line represents the probable anther length for each plant at the time of the last pre-meiotic telophase and serves as a guide in separating autoradiographs due to meiotic from those possibly due to previous mitotic DNA synthesis. Only the autoradiographs of the youngest bud of plant No. 3 and the two youngest buds of plant No. 14 fall into the latter category. The time taken for P^{32} to reach the buds after immersion of the inflorescences in the active solution (two to three hours, according to TAYLOR, 1953) has not been taken into account.

radiography. Using relatively high P^{32} concentrations and short uptake periods he showed that DNA synthesis takes place during a fairly short period in late pre-leptotene interphase, but found no evidence for further DNA synthesis until the interphase before microspore mitosis.

The P^{32} investigation presented here is comparable to the photometric analysis of SWIFT with respect to the stages of meiosis studied, the results, however, differ in the suggested time of the DNA increase. This may or may not be attributable to differences between *Lilium* and *Tradescantia*. The data of OGUR *et al.*, although derived from material of the same genus, do not cover early meiosis; where they do coincide in developmental stage with those given here, there is no agreement:

I have found no evidence for DNA synthesis during the period of increase reported by OGUR.

The conclusions on meiotic DNA synthesis briefly given here are in essential agreement with those of TAYLOR. My data are as yet insufficient for a meaningful discussion of the time interval during which the pre-leptotene synthesis takes place. Experiments now being planned should overcome this lack. The two autoradiograph studies are complementary in two aspects: the longer P^{32} exposure periods used in the present investigation, while resulting in a loss of precision in the establishment of the exact time and duration of synthesis, form stronger negative evidence against a gradual increase of DNA during meiosis proper as suggested by the microchemical analyses; the fact that considerably lower P^{32} dosages led to the same results tends to rule out the possible criticism of excessive radiation damage in TAYLOR's experiments. One point on which the two investigations do not agree is the pachytene incorporation of P^{32} into a non-labile cytoplasmic component shown by TAYLOR in *Lilium longiflorum*. I have not observed any indication of this phenomenon. It is possible, however, that my P^{32} dosages were too low to show this incorporation.

Some discrepancies between P^{32} labelling results and those obtained through chemical or photometric analysis are perhaps to be expected if polymerization of nucleotides to DNA can occur without incorporation of phosphorus. At the present time, however, it is quite obvious that more data which can be directly compared are needed before generalization can be attempted profitably. It may be well to keep in mind the suggestion that meiosis is dependent upon a supply of materials secreted by the cells surrounding the developing microsporocytes, or spermatocytes (COOPER, 1952; MONTALENTI *et al.*, 1950). Such transfer phenomena could have a strong bearing on the DNA metabolism of the sporogenous cells.

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BIOLOGICAL EFFECTS OF X-IRRADIATION AT LOW TEMPERATURES

BY *N. NYBOM, U. LUNDQVIST, Å. GUSTAFSSON,* AND *L. EHRENBERG*
SVALÖF AND STOCKHOLM

IN a recent paper by NYBOM, GUSTAFSSON, and EHRENBERG (1952, pp. 356—358) it was shown that the immediate X-ray injury is greatly influenced by the temperature prevailing at the time of irradiation. Dormant seeds, with an average water content in the embryo of ten per cent, stand temperatures down to that of liquid air, circa — 190° C, without losing their germination capacity or decreasing the consequent growth. In the experiments to be reported here the low temperature effects have been analysed in considerable detail, covering also the mutation rates of the second generation. The properties examined are the following:

Immediate irradiation effects — germination capacity; the growth rates of seedlings up to three or four weeks after the time of sowing (greenhouse tests); the amount of chromosome disturbances in roots of germinating seeds.

Field properties of the first generation — sprouting ability two to three weeks after sowing; number of surviving plants at harvest time; average fertility of the harvested plants, measured as the proportion of fertile to total number of florets per spike and plant; the percentage of sterile plants having a fertility of less than 90 per cent.

Second generation effects — number and types of chlorophyll mutants (greenhouse tests).

The methods of irradiation are briefly described in the above-mentioned paper of NYBOM *et al.* (p. 356). Dormant seeds of the variety »Bonus» were kept at a temperature of — 190° for one hour before and after, as well as during the irradiation. Simultaneously seeds were treated at the temperature of +20°. The seeds were uniform and medium-sized owing to a sifting before the treatment. The water content at the time of irradiation was circa 11 per cent.

During the irradiation test tubes containing the seeds were immersed in thermos flasks, one with liquid air, one with water of 20° C. The dose measurement was performed with a Victoreen type dosimeter

in the test tube of the 20° flask. Since the density of liquid air during its ageing, *i. e.* under a prevalent evaporation of nitrogen, increases from 0,9 to 1,1, the uncertainty in the dose given due to this difference in density was determined. Dose measurements performed in both flasks with compounds of different density (air, water, ethanol, glycerol) demonstrated that the doses given at both temperatures were equal within 1,5 per cent.

The temperature of liquid air is chosen as — 190°, *i. e.* the average of the temperature of fresh liquid air (— 194,5°) and that of aged liquid air, *i. e.* chiefly oxygen (— 185°).

IMMEDIATE EFFECTS.

In addition to the data given by NYBOM *et al.* (*loc. cit.*) we here mention the following results with regard to the growth rate under greenhouse conditions (Table 1).

TABLE 1. *Growth rates in greenhouse tests.*

Series	+20°C		−190°C	
	Leaf-length, mm 14 days	No. of germinated seeds (100 sown)	Leaf-length, mm 14 days	No. of germinated seeds (100 sown)
Control 1	156,7 ± 1,5	99	167,1 ± 1,8	98
Control 2	154,1 ± 1,8	98	161,3 ± 4,3	99
10000 r	102,3 ± 2,3	100	148,3 ± 2,1	99
20000 r	41,0 ± 2,0	56	105,0 ± 2,4	96

Evidently a dose of 20000 r with the irradiation temperature of — 190° is about as effective as half this dose at +20°, both with regard to germination and growth.

In their paper of 1941 FRÖIER, GELIN and GUSTAFSSON, as well as GELIN, 1941, gave some results relating to the chromosome damage of diploid and polyploid cereals arisen after irradiation. The analysis of the chromosome disturbances induced at temperatures of +20° and — 190° was carried out in a similar manner. Irradiated seeds were put for germination in Petri dishes and their roots fixed 24—48 hours after the beginning of the soaking when circa 10 mm long. The occurrence of deleted chromosomes (fragments) and chromosome translocations (bridges) was noted. The analysis deals with the first mitotic cycle when germination has just started. The results are presented in Table 2.

TABLE 2. *Induced chromosome disturbances at different temperatures.*

Dose in r-units	+20°C				−190°C			
	% disturbances	% bridges		Sum of examined cells	% disturbances	% bridges		Sum of examined cells
		of all cells	of disturbed cells			of all cells	of disturbed cells	
5000	13,4	2,9	21,6	350	7,6	3,1	40,8	353
10000	10,4	3,0	28,8	67	10,8	3,5	32,4	369
15000	33,0	12,4	37,6	315	24,2	7,2	29,6	181
20000	50,0	18,6	37,2	86	32,1	6,2	19,3	274

With the exception of the +20° and 10000 r-series, where just a few divisions were found, there is a striking difference between the two sets of experiments, the low temperature series consistently being less affected. For the two highest doses this holds true both with regard to the total percentage of disturbances and the percentage of bridges. With rising dose the percentage of cells containing bridges, if calculated per disturbed cell, distinctly increases from 22 to 38 per cent in the case of +20°. On the other hand, in the case of —190°, the corresponding values fall from 41 to 19 per cent. The number of bridges per cell with bridge-formation is, however, rather constant, possibly a little lower in the liquid air experiments, where it varies from 1,2 to 1,4, as against 1,3 to 1,5 at the higher temperature.

FIELD PROPERTIES OF THE FIRST GENERATION.

The germination rates, three weeks after the sowing, and the number of mature plants are found in Table 3.

There is consequently no doubt that the low temperature counteracts the cell and seedling lethality, insofar as more plants sprout and reach maturity. This is especially noticeable with high dosages. In this connection the decreased viability of the unirradiated control at —190° should be pointed out (*cf.* NYBOM *et al.*, Fig. 19).

Of great interest are the data on average plant and spike fertilities (Table 4).

The mean plant fertilities are based on 50 plants in the case of controls and 100 plants in the X_1 series, except in the case of +20°, 15000 r, where no more than 18 plants were available, and —190°, 15000 r, where 170 plants were examined. The X_1 plants gave on an average 2—3—4 spikes, all of which were analysed.

TABLE 3. *Sprouting ability in the field and mature plants at harvest.*

Dose	Temperature	No. of seeds sown	Sprouting ability (% germination after three weeks)	Mature plants at harvest (% of seeds sown)
Control	+ 20° C	300	93	84
»	— 190°	»	95	69
5000 r	+ 20°	»	83	73
»	— 190°	»	83	69
10000 r	+ 20°	»	54	55
»	— 190°	»	*74*	*76*
15000 r	+ 20°	600	5	5
»	— 190°	»	*39*	*32*
20000 r	+ 20°	»	0,5	—
»	— 190°	»	*2,0*	—

There is a consistent evidence (P in all instances below 0,001) that the liquid air temperature actually protects against radiation-induced sterility. Since chromosome aberrations, arisen in the irradiated seed materials, are chiefly responsible for the occurrence of sterility (*v.* KAPLAN, 1951), we may safely conclude, in addition to the evidence from the examined immediate effects, that low temperatures protect against chromosome conditioned gamete and embryo lethality. This implies that the number of chromosome disturbances reaching meiotic and postmeiotic stages is less frequent with low than with normal temperatures. Comparing the results of 10000 and 15000 r, we notice that the liquid air series require a 50 per cent higher dose in order to produce the equivalent damage.

In his papers of 1947 and 1951, KAPLAN used the percentage of ± sterile X_1 plants as an indication of chromosome disturbances. For the sake of completeness we may here state that the occurrence of sterile plants, with a fertility less than 90 per cent, is also distinctly decreased after an irradiation at low temperatures.

SECOND GENERATION BEHAVIOUR.

In order to determine the frequency of induced mutations the spikes of the X_1 plants were germinated in moist sand in the greenhouse. After 2—3 weeks, when the seedlings reached a height of 8—12 cm, the number of spikes containing pigment deficient mutations

TABLE 4. *X_1 fertilities and X_2 mutation rates.*

Dose	Temperature	Mean plant fertility %	Mean spike fertility %	Mutation rate		Mutation rate		No. of X_1 plants	No. of X_1 spikes
				per plant progeny %	per plant progeny and r-unit	per spike progeny %	per spike progeny and r-unit		
Control	+ 20° C	97,9	98,7	0,0	0,0	0,0	0,0	50	151
»	— 190°	97,8	98,6	0,0	0,0	0,0	0,0	50	206
5000 r	+ 20°	82,3 ± 1,2	81,6 ± 1,0	11,7	$23,4 \cdot 10^{-6}$	4,7	$9,4 \cdot 10^{-6}$	214	530
»	— 190°	87,8 ± 0,9***	86,7 ± 0,8***	6,0*	$12,0 \cdot 10^{-6}$	2,2**	$4,4 \cdot 10^{-6}$	199	594
10000 r	+ 20°	51,5 ± 2,4	51,8 ± 1,9	24,4	$24,4 \cdot 10^{-6}$	9,1	$9,1 \cdot 10^{-6}$	82	221
»	— 190°	70,7 ± 1,4***	70,7 ± 1,2***	18,2	$18,2 \cdot 10^{-6}$	6,8	$6,8 \cdot 10^{-6}$	198	528
15000 r	+ 20°	27,2 ± 4,9	30,3 ± 3,9	10,0	$6,7 \cdot 10^{-6}$	4,6	$3,0 \cdot 10^{-6}$	10	22
»	— 190°	52,0 ± 1,5***	53,6 ± 1,1***	29,2*	$19,5 \cdot 10^{-6}$	9,6	$6,4 \cdot 10^{-6}$	144	501

were noted and the mutations were classified according to the scheme of GUSTAFSSON (1940).

The controls have produced no single case of chlorophyll mutation (Table 4).

For 5000 and 10000 r the mutation rates are lower with the liquid air treatments, both when plant and spike progenies are considered. With 15000 r the situation is reverse. Owing to the high standard error in the case of 15000 r and $+20°$, the difference between the two sets of material is less significant but still gives a probability of circa 0,05 for the plant progenies.

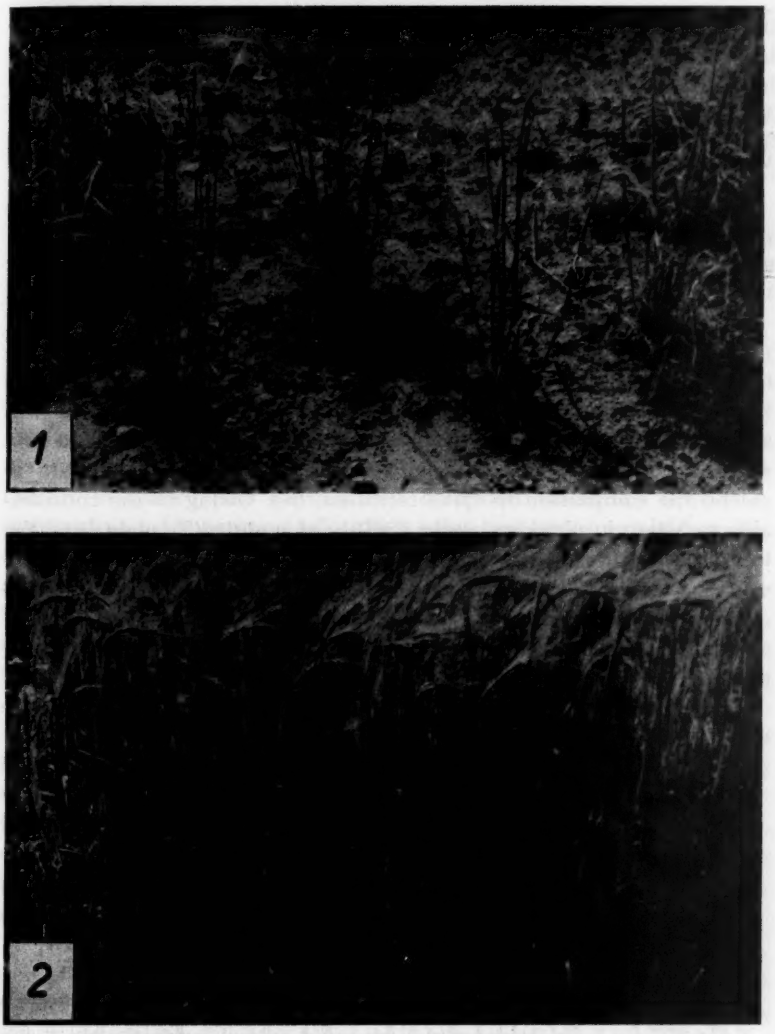
If we calculate the mutation rate per r-unit and plant, respectively spike progeny, we find that the normal temperature series show approximately constant values at 5000 and 10000 r and a conspicuous drop at 15000 r. This result agrees with previous data, published by GUSTAFSSON, 1947; MAC KEY, 1951; EHRENBERG *et al.*, 1952, and is explained by processes of elimination occurring during ontogenesis, an »intrasomatic selection» according to KAPLAN (1951). Evidently this selection is diminished or displaced towards higher doses after irradiation at the liquid air temperature. In no case do the liquid air series reach the maximum level of the normal temperature series. Also if averaged the mutation rates display a certain discrepancy: 18,1 as against $16{,}6 \cdot 10^{-6}$ per plant progeny, and 7,2 as against $5{,}9 \cdot 10^{-6}$ per spike progeny.

The conclusion is that also with regard to mutation rate the low temperature treatment exerts a protective action.

In a general paper to be published shortly we shall analyse in more detail and on larger material the ways in which the above-mentioned elimination proceeds. Here we want to emphasize that the findings of this investigation agree with the results based on the much larger material.

Table 5 indicates that the maximum rate of mutation appears in the progenies of mother plants having a moderately depressed fertility, with $+20°$ between 30 and 90 per cent, in the liquid air series between 30 and 80 per cent. The very fertile and the very sterile X_1 plants produce a low number of mutations, the former owing to the fact that they arose from slightly or not at all affected seeds, the latter ones owing to the low number of kernels formed per spike and plant but, in addition, owing to the pronounced elimination of mutated cells.

That there is some sort of »intrasomatic selection» or elimination is further indicated by Table 6. Here the mean fertilities of the X_1 plants



Figs. 1—2. — Parallel experimental plots illustrating the lethal effect of 15000 r on seeds at +20° C (Fig. 1) and at —190° C (Fig. 2).

TABLE 5. *Mutation rates in progenies of differently fertile X_1 plants.*

X_1 fertility %	0 — 30	— 40	— 50	— 60	— 70	— 80	— 90	— 100	Σ
+ 20° C									
No. of X_1 plants	21	16	10	23	18	38	27	30	= 183
No. of mutants	1	6		8		6	4	3	= 28
Mutation rate %	4,8	23,1		19,5		15,8	14,8	10,0	15,3 %
— 190° C									
No. of X_1 plants	16	17	39	50	48	55	57	58	= 340
No. of mutants	1	10		27		13	6	1	= 58
Mutation rate %	6,3	17,9		27,8		23,7	10,5	1,7	17,1 %

producing chlorophyll mutants are compared with the fertilities of the non-mutated plants. Similarly the fertilities of the X_1 spikes producing mutants have been computed. In Table 4, columns 3 and 4, it was shown that there are no differences between the average plant and spike fertilities in irradiated material. It is therefore legitimate to extend the comparison to spike fertilities, too. Owing to the considerable variation in plant and spike fertility of mutated X_1 plants and the low number of mutants analysed, the standard errors are high. Nevertheless there are distinct indications of the following regularities:

(1) The mean fertility of the mutated plants of both series (— 190° and +20°) lies circa 7 per cent higher than that of the non-mutated plants ($0{,}2 > P > 0{,}1$).

(2) The mean fertility of the mutated spikes is 13 per cent higher than that of the non-mutated plants ($P = 0{,}05$).

TABLE 6. *Average fertilities of plants and spikes producing or not producing mutants.*

1	2	3	4	5		
Series	Fertility of non-mutated X_1 plants %	Fertility of mutated X_1 plants %	Fertility of mutated X_1 spikes %	3—2 %	4—2 %	4—3 %
+ 20°, 5000 r	81,1 ± 1,4	87,1 ± 3,1	87,8 ± 5,2	+ 6,0	+ 6,7	+ 0,7
+ 20°, 10000 r	48,8 ± 2,9	54,1 ± 3,8	60,7 ± 7,3	+ 5,2	+ 11,8	+ 6,6
+ 20°, 15000 r	22,8	47,5	59,0	+ 24,7	+ 36,2	+ 11,5
— 190°, 5000 r	87,8 ± 0,9	87,1 ± 3,6	88,4 ± 4,8	— 0,7	+ 0,6	+ 1,3
— 190°, 10000 r	70,5 ± 1,6	69,4 ± 3,1	78,9 ± 6,6	— 1,1	+ 8,4	+ 9,5
— 190°, 15000 r	50,0 ± 1,8	56,8 ± 2,2	64,1 ± 3,4	+ 6,9	+ 14,0	+ 7,2
Average	—	—	—	+ 6,8	+ 13,0	+ 6,1

(3) The mean fertility of the mutated spikes is by circa 6 per cent higher than that of the mutated plants ($P = 0{,}02—0{,}01$). In the general paper to be published shortly we shall point out that the intra-plant selection is less marked with albina than with other types of mutation. The material of the present experiments does not allow of a refined analysis. The figures just reported show, however, that for the chlorophyll mutations as a whole there is a considerable selection at work.

(4) The elimination, as evidenced in Table 6, column 5, is less marked in the $-190°$ treatments than at normal temperature, in agreement with the postulated direct action of X-rays at liquid air temperature.

DISCUSSION.

Genetical and physiological results. — Already in his paper of 1931, STADLER presented some data concerning the effects of low temperature at the time of irradiation. He found that the mutation frequency in barley was considerably decreased when the irradiation occurred at a temperature of $-80°$ (seeds imbedded in solid carbon dioxide) in comparison with room or higher temperatures. The mutation yield was approximately $^1/_3$ lower, if calculated per r-unit. The seedling growth, too, was distinctly better. The maximum roentgen dose tolerated at $-80°$ was about $^2/_3$ higher than at 23°. In this case, however, the possible influence of carbon dioxide was not considered.

MAXWELL and KEMPTON (1939), as well as KEMPTON and MAXWELL (1941), and MAXWELL, KEMPTON, and MOSLEY (1942), found in their extensive work with dormant maize seeds that the percentage of survival increased for the temperatures of 27°, 0°, $-66°$, and $-187°$ in the order mentioned and that a similar order of insensitivity prevailed with regard to seedling growth. A pronounced point of insensitivity was found with an irradiation temperature of 50—60°, too. The maximum sensitivity lay in the region of 0° to room temperature.

FABERGÉ (1950) and RAJEWSKY (1952) have compared the irradiation effects on pollen grain development at different temperatures. The former author stated that at $-190°$ C the sensitivity of the chromosomes, as measured by the number of breaks observed in pollen tube mitosis of *Tradescantia*, was about one fifth of that at $+25°$. The curve of H_2O_2 production, as plotted against temperature, greatly resembles the curve of chromosome sensitivity. The H_2O_2 production, however, stops completely below $-116°$, whereas chromosome breaks are still produced at $-190°$. X-ray action, the author concludes, may

still be usefully regarded as particulate, and a target theory is still possible, but a factor must be added to take into account the spread of effect around ionization columns. Similarly, RAJEWSKY found a consistent protection by liquid air treatments against X-irradiation in pollen tube growth of *Digitalis*, with doses ranging from 0 up to 600000 r-units.

With the express purpose of discriminating between direct and indirect irradiation effects, KAPLAN (1947, 1951) made a series of experiments in barley comparing the results with temperatures of $-65°$ and $+17{,}5°$. He examined two properties of interest in connection with our study: (1) the number of $\pm$ sterile X_1 plants appearing after the application of 6000 r, and (2) the number of recessive mutants in the subsequent X_2 generation. He concluded that the low temperature did not influence either property. For dormant barley seeds, consequently, there is no diffusion or spreading of the energy involved in chromosome breakage or factor mutation. An analysis of his original data, however, shows that there is a small but distinct surplus of sterile as well as mutated first generation plants in the case of $+17{,}5°$ as compared with $-65°$.

This investigation has evidenced that KAPLAN's conclusions do not hold true for the still lower temperature of $-190°$ at the time of irradiation and that, in addition, his conclusions oppose the previous results obtained already by STADLER (*loc. cit.*). The liquid air treatments rather exert a protective influence in all properties examined, in germination, sprouting ability, chromosome disturbances, percentage of sterile plants, average plant and spike fertility, and mutation rate.

Chemical interpretations. — The chemical effect of X-rays on a solution may be due to an indirect action [in the case of water through radicals H, OH and, in the presence of oxygen, HO_2 (WEISS, 1947)] or a direct action on the solvent molecules:

$$R \rightarrow R^+ + e^-.$$

In the latter case the ion R^+ is eventually changed to the state which is energetically most probable. The first step of the energy absorption, *i. e.* the electron projection, is not influenced by temperature. Those secondary reactions, however, the rates of which are determined by the rate of diffusion of molecules or by chemical reactions requiring an activation energy, give a decreased yield when the temperature is lowered. If the temperature decrease involves a change from liquid to solid state, the diffusion rate is extremely diminished. Low tem-

peratures will consequently cause a decrease of the reaction yield in the case of an indirect action of the radiation. LEFORT (1950 b) has found that in a dilute solution of K I the oxidation of I^- to I is diminished to about $^1/_3$ upon a temperature decrease from $+20°$ to $-4°$ C, which implies a solidificaton of the solution. A further lowering of the temperature to $-196°$ C causes an additional decrease of the yield with about 30 %. The effect is obtained with X-rays as well as with α particles. At the low concentration in question (about 0,001 M) LEFORT has demonstrated the reaction to be chiefly indirect. At higher concentrations (about 1 M), however, where a direct radiation action prevails, the decreasing effect of the solidification as well as of the further lowering in temperature is much less obvious. Similar effects have been observed as regards the decomposition of sodium arsenite (*cf.* LEFORT, 1950 b) and ferrous sulphate (MINDER and LIECHTI, 1946) in water solution. In the latter case the reaction yield is independent of temperatures between $+4°$ and $+54°$ C, whereas frozen solutions are not oxidized at all. The formation of H_2O_2 in ice containing O_2 is also decreased when the temperature is lowered (LEFORT, 1950 a). The reduction of methylene blue in water-free glycerol solution is decreased upon freezing the glycerol, only leaving the direct radiation effect (ZIMMER and CRON, 1944; *cf.* TIMOFÉEFF-RESSOVSKY and ZIMMER, 1947).

In all the examples mentioned, the change of the physical state strongly alters the diffusion rate. In the solid state the prevailing reaction becomes a recombination of the radicals very close to their formation sites. Since the activation energy for the recombination $H+OH \rightarrow H_2O$ is said to be zero (LEA, 1947), a temperature lowering without a change of state will make the recombination prevail in cases where the secondary reactions of these radicals with solute molecules require an activation energy.

In the case of dry dormant barley seeds, the physical state is not easily defined. In the germinating embryo, which contains about 70 % of water, the protoplasm is best described as a sol. Compared to this the protoplasm of the dormant embryo cells, containing only about 10 % of water, is certainly a rather dry gel. Probably part of the difference in the radiation sensitivity between germinating and dormant seeds may be due to this difference in state (NYBOM *et al.*, 1952). No doubt, the freezing of dormant seeds in liquid air makes them still more solid, thus further inhibiting diffusion processes.

It can be concluded that the radiation protection obtained with

liquid air clearly indicates that, regarding the biological effects examined, part of the chemical reactions behind these effects are due to an indirect action of the radiation.

This conclusion is further confirmed by experiments involving the irradiation of dormant seeds in an atmosphere containing H_2S (NYBOM *et al.*, 1952, who present data concerning the protective influence of H_2S against a retardation in growth). Similar figures concerning sterility and mutation rates will be presented elsewhere. The protective action of H_2S is no doubt due to its reaction with free oxidizing radicals, especially HO_2, before these are able to interfere with the various biological processes.

In this connection the studies by SETLOW and others (*cf.* the review in POLLARD, 1953) should be considered: When pure, dry preparations of viruses and enzymes are irradiated, a certain increase of the radiation stability is found at low temperatures. The altered sensitivity is interpreted as due to changes in the condition of the molecules.

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SUMMARY.

This paper evidences that an X-ray irradiation is less injurious at $-190°$ C than at $+20°$. This holds true for a series of different properties, including growth conditions, mitotic chromosome disturbances, and mutation rates. The low temperature acts as a protection against physiological as well as genetical X-ray damage. It eliminates most or part of the indirect radiation effects.

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X-RAY PHOTOGRAPHY AND SENSITIVITY IN FOREST TREE SPECIES

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IN this paper, being of a preliminary character, the authors deal with two problems. The first concerns the seed control in forestry and forest genetics, how to determine on an early stage, quickly and definitely, whether a certain seed sample is sufficiently good to warrant its wide use in practice. For this purpose a method has been worked out, applying X-ray photography. The second problem concerns the behaviour of forest tree seed after irradiation, the subsequent plant development, the recovery or death of the growing irradiated individuals, and the formation of chimaeras by means of induced vegetative mutations. With the exception of irradiated fruit tree materials (GRANHALL, 1953), chiefly branches and buds, there has been no long run study of the X-ray action on long-lived organisms. The two problems just mentioned hook into each other owing to the necessary object of finding a method of photography that does not damage the seed and does not markedly induce lethal mutation or chromosome disturbances.

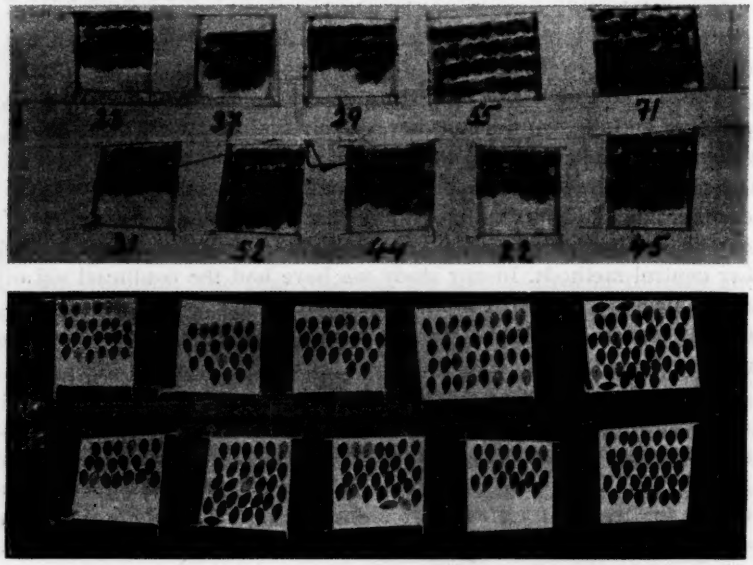
In his publication of 1951, HUSS gave a detailed account of the seed control methods used in silviculture for the analysis of a certain seed sample, its properties and quality. Extensive experiments of hybridization and inbreeding in various forest tree species are continually being carried out at the Genetics Department. They result in valuable seed materials, the analysis of which requires more refined methods of study than available up to now. So, for instance: it is necessary to determine in an exact way the amount of empty *contra* full seed of a certain sample and the mutual frequency of these two types of seed after different kinds of pollination. The seed cleaning apparatus, worked out by TIRÉN for other purposes (*cf.* HUSS, *l. c.*) allows of an approximation, no definite analysis. A refined seed control would no doubt be of value for the practice, too, especially if it permits the study of seed development at an early time and the tedious sectioning and germination analysis can be simplified or made auxiliary. The idea was then close at hand to determine the percentage of empty or full seed by means of X-ray photography, to establish the final state of

development in this way, to settle whether there are or are not embryos in the seed, although endosperm is present, or, finally, to measure the absolute and relative length of the embryo.

The method of X-ray photography, described here, must not be considered definite. On the contrary, there remains a series of technical improvements to be done. In addition, its possibilities must be carefully checked in relation to the more traditional seed control methods. On the other hand, X-ray photography has already given results of theoretical value and forms, no doubt, a valuable complement to the other control methods. In our study we have had the continual aid of the technical staff of AB GEORG SCHÖNANDER, Stockholm, and we extend our sincere thanks to Mr. E. WASSER, Director of the firm, to Dr. K. FENZ and Mr. L. KINDEL.

The equipments of seed photography are based on an instrument for contact therapy, model SCHÖNANDER, TEA-25, consisting of a high voltage aggregate on a mobile stand with holder for the X-ray tube in a shield. The X-ray tube is enclosed in oil and possesses a beryllium window for transmitting the soft X-radiation. The shield is 160 mm long, its largest diameter 85 mm, and has a total weight of 1,15 kg. It is easily turnable in all directions. The X-ray tube produces so-called Grenz-rays, very soft X-rays, and is rated for low voltages, less than 25 kilovolts and for maximally 10 milliamperes continuously. This type of roentgen radiation is readily absorbed by thin sections of low atomic numbers and specially suited for photography. Different kinds of roentgen films have been tried. It is of course necessary to have a fine-grained film emulsion that produces high contrast and shows the very fine details of the object. The distance from focus has been varied between 15 and 35 centimetres.

The detail analysis of a seed portion is carried out in such a way as illustrated in Fig. 1. All seeds of a cone, either full or empty, are fastened to a strip of tape, placed on a sort of paper board containing round or four-edged holes. The seeds adhere to the tape without being damaged and may after the photographing be released and examined seed after seed. In this way it is possible to control every seed of the cone or sample with regard to its internal state, especially its embryo development, and to relate it to the germination capacity, sprouting ability, number of cotyledons of the seed in question, as well as the consequent growth of the seedling or — if this is considerable desirable — to section the seed and to determine the embryo length according to routine methods. If just the percentage of empty seeds should be



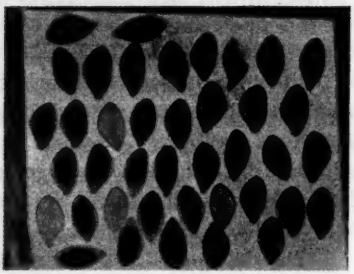


Fig. 1 *a*. Cardboard model with seeds pasted on tape strips. Scot's pine (*Pinus silvestris*). Provenance Sweden: Bogesund, near Stockholm, 1951: 59, tree 1. The figures denote different cones. All seeds of a cone have been pasted. — Fig. 1 *b*. X-ray photograph of the same seed portions as in 1 *a*. 10 mA, 8 sec., 12 kV, 25 cm. focal distance. — Fig. 1 *c*. Detail picture of cone 71. Light seeds are empty, lacking embryo and endosperm.

determined, a simpler mode of photography can be applied. The seeds may in such a case quite simply be laid on the film holder itself and then photographed. In pine it is desirable to place the seeds with the broad-side up. The narrow side being turned upwards, the embryo details will be less distinct.

The forest tree species, examined so far by this method, are *Alnus glutinosa* and *incana*, *Pinus silvestris*, *Picea Abies*, *Larix sibirica*, and *Thuja plicata*. All species permit a close study of the seed properties. The methods have been specially worked out for the Scot's pine. Here mixed and specified seed samples have been analysed, of native or

foreign extraction, large or small seed, full or empty, hybrid and inbred seed, or seed obtained after open pollination. Some photographs are reproduced here, indicating the usefulness and fine details of the method. Exposure time and mode of treatment are given in the figure texts. Within short a close analysis will be published concerning the development of hybrid and inbred seed and in addition dealing with a series of properties of the mother trees used in crossing (PLYM-FORSHELL). Most clear photographs have been obtained with Ilfex roentgen film, 12 kV, 10 mA, 4—8 seconds of exposure, 25 centimetres distance from the focus, and so-called roentgen development. The embryo length, as well as other embryo and endosperm characters, can be easily settled from the negative under low magnification. If no precise measuring is necessary, the details can be studied quite simply with a pocket lens.

The X-ray photography has permitted the analysis of the following seed properties: (1) The percentage of empty seeds. These contain neither endosperm nor embryo and correspond to the seed type O in KUJALA's classification of conifer seed development (1927). (2) The percentage of seeds with endosperm but without embryo (KUJALA's seed type IA). (3) The percentage of seeds with endosperm as well as embryo, the embryos showing a different stage of development (seed type IB to V according to KUJALA), *i. e.* from poorly filled to fully ripe and well-developed seed. (4) The detection of different seed abnormalities, for instance turned, twisted or split embryos, polyembryony, *etc.* (5) The length of the embryo, as measured from the tip of the radicula or the micropylar region to the extreme end of the more or less well-developed cotyledons.

According to WIBECK (1929), there is in Scot's pine a pronounced correlation between the average length of the embryo and the germinability of the seed. He fixed what is called the embryo relation, *i. e.* the proportion between length of embryo (K1) and endosperm (S1), and found that the germination rate of a seed sample runs parallel to the number of seeds having an embryo relation of 65—70 per cent. Light, temperature and humidity certainly influence the germination profoundly, but possibly there is a certain minimum threshold of embryo development which the seed must attain in order to germinate normally. With X-ray photography the value of a seed sample can be *rapidly* settled without the aid of any sectioning or germination in the laboratory. This is specially desirable in years like 1952, when the pine seed in many regions of northern Sweden, although cone formation

was abundant, developed poorly and gave a low germination rate. It is a necessary object to work out a reliable test of the seed quality early in the fall, district after district, high above or at sea level, in north-slopes or in sun-exposed areas, in the Arctic zone or more towards south. A series of individual tests have been started in this manner, relating to natural conditions or to grafted materials.

This communication has a sort of celebration character. It is exactly fifty years since A. N. LUNDSTRÖM, then Professor of Uppsala University, stated in a meeting of the Silvicultural Society of North Sweden (Föreningen för skogsvård i Norrland) that he had examined the seed setting of forest trees by means of X-ray photography. He said (1903, p. 15, in translation): »In order to decide whether cones from certain districts ought to be collected for seeding, the speaker had used roentgen rays. Some seeds then appeared dark, others light. The latter ones showed themselves to be incompletely filled and to contain more or less air; they were dull. This method could not be used in practice, however, since it would be too tedious and troublesome.» This important report was entirely forgotten and the possibilities of the method neglected until this study began a year ago. In 1943, FREISLEBEN, MÜLLER and SENGBUSCH briefly announced a study of fruit and seed details in horticultural plants (tomatoes, citrus-fruits, grapes), as well as of wood structure by means of X-photography. Recently the General Electrics firm of Milwaukee, USA, has constructed an X-ray equipment for the identification of infested grain, the extent of infestation, the stage that infestation has reached, and the ability or inability of the insects to live and to reproduce. How widely this equipment is now used in agriculture, we do not know.

The study of X-ray sensitivity of forest tree seed was started for several reasons: (1) in order to determine the X-ray dose producing lethalizing effects in first generation growth as a control of the dose effects in photography, (2) to study and follow for a long sequence of years the roentgen morphoses, their continued presence, slow disappearance or successive destruction of the plant, (3) to produce and to gather vegetative mutations, in various properties like pollen sac colour or seed details. In this connection it is well worth mentioning that chlorophyll lethals may reach a surprisingly high frequency in many populations, measured by the frequency of recessives in average stand or individual tree progenies. Mr. V. EICHE of the Genetics Department has found gene frequencies of chlorophyll lethals amounting to 0,1 or even more, up to 0,3. This last figure cor-

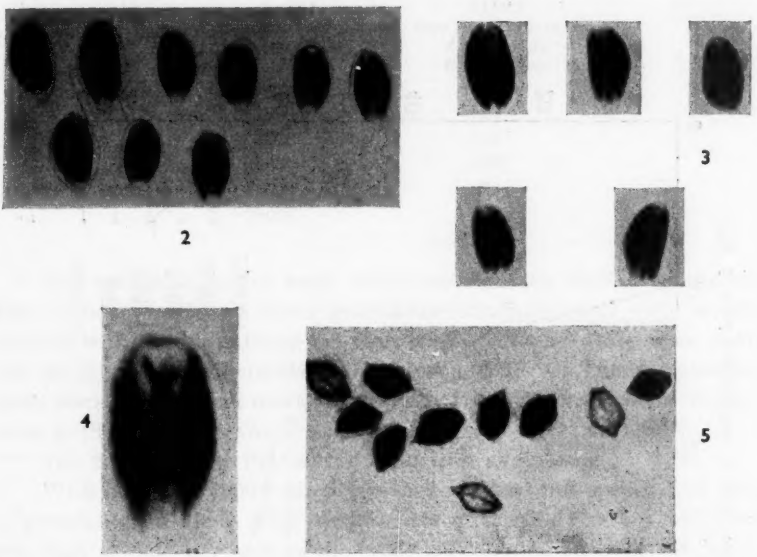


Fig. 2. Seeds with embryo details. Scot's pine, provenance Norway: Tranöy. 10 mA, 10 sec., 12 kV, 35 cm. focal distance. — Fig. 3. Five seeds with embryo details. Spanish provenance, Scot's pine. 10 mA, 3—4 sec., 12 kV, 25 cm. focal distance. — Fig. 4. Seed of the same provenance. -- Fig. 5. Seed of alder (*Alnus glutinosa*). Full and empty seed.

responds to a heterozygote number of 20—40 %. These heterozygotes are of extreme value for the production of homozygous mutations, growing and parasiting on the normal tissue, or even able of propagation by means of grafting, and for the successful study of neutron, X-ray and radioactive isotope effects on individual genes and their mutation rates with different ion density.

The material used in this study consisted of pine, spruce and alder seed (*Pinus silvestris*, *Picea Abies*, *Alnus glutinosa*), from Sweden: Västmanland, Sweden: Uppland and Sweden: Scania. The germination percentage, in the Jacobsen apparatus, was 88, 76 and 97, respectively. In a first series 100 seeds of each species were given from 200 up to 25000 r-units and were sown in two replications, each comprising 50 seeds. In a second series with three replications pine and spruce seed of the same origin were given from 75 to 900 r. The doses were the following:

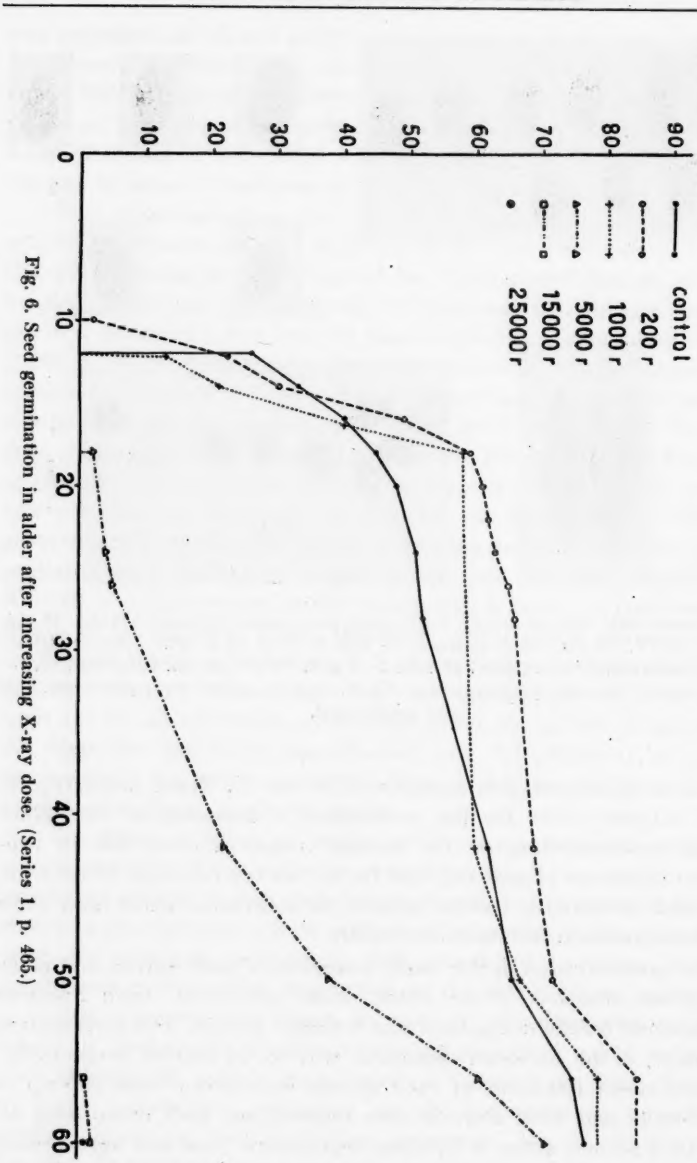


Fig. 6. Seed germination in alder after increasing X-ray dose. (Series 1, p. 465.)

Series 1	*Series 2*
Pine, spruce and alder seeds.	Pine and spruce seeds.
2 replications	3 replications
175 kV, 7 mA	175 kV, 15 mA
Control	Control
200 r	75 r
1000	150
5000	300
15000	600
25000	900

The pine and spruce seeds were sown exactly half a centimetre deep in boxes with soil under greenhouse conditions and were loosely covered with sand. Similarly the alder seeds, although these were sown less deeply. The temperature centred around 15° C. The germinating seeds were counted and marked every day from the onset of the experiment to the 30th day. The final checking was made after 60 days.

The results of the first series are briefly as follows:

With 5000 to 25000 r no single seed of pine and spruce was able to germinate. In alder (Fig. 6) there are after 5000 r and 60 days no less than seventy per cent of the seeds germinated, although the seedlings remain weak and are in many instances not able of survival. The curve of germination is in this case distinctly altered. With 15000 and 25000 r very few alder seeds germinated. With 200 r there is in alder a distinctly beneficial effect of the radiation. In pine and spruce no such effect was obtained. With 1000 r there are in *Pinus silvestris* a fairly high number of germinating seeds (*circa* 30 per cent) but the seedlings were weak and were suddenly killed by *Fusarium* attacks. In spruce there is a very low germination rate after 1000 r. Already with 200 r there is a pronounced decrease in germination and viability of both species.

Series 2 was started in order to obtain some detail information concerning the dose dependence. Seeds were sown in the same manner as in the first series and the germination was followed day for day. In pine seeds a dose of 900 r exerted a distinctly detrimental effect (Fig. 7). Less evident was the effect with 600 r. Doses of 75 to 300 r give germination curves essentially similar to the control material.

In spruce seeds the dose of 600 and especially of 900 r distinctly decrease germination (Fig. 8). The control and the 300 r series are similar in behaviour. With 75 and 150 r a certain stimulating effect is noticeable.

A further analysis was made in order to analyse the X-ray de-

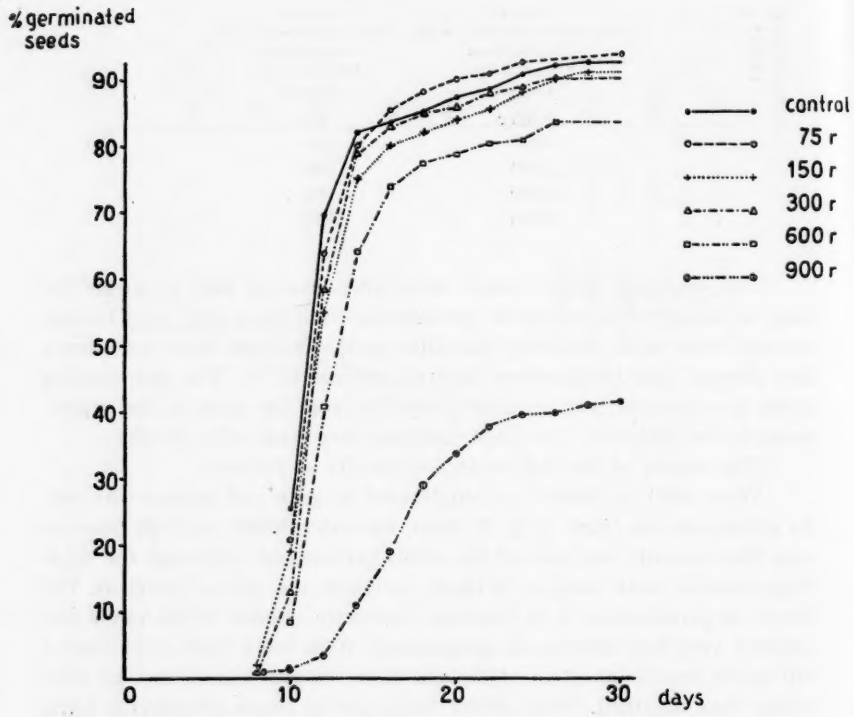


Fig. 7. Seed germination in Scot's pine after increasing X-ray dose. (Series 2, p. 465.)

pendence. 2×50 seeds were sown in the Jacobsen apparatus on filter paper, and the mean plant weight was determined 10 days after the onset of experiments. The results are illustrated in Fig. 9. In pine there is a slight stimulation effect with 75—300 r and a definite decrease in growth with 900 r. In spruce no stimulation is encountered in this case, and a distinct decrease of growth is noticeable with 600 r.

This study has shown that the seeds of the three species examined react differently to X-ray irradiation, the least sensitive seeds being those of alder, the most sensitive being those of spruce. The killing dose lies in alder above 5000 r, in pine above 1000 r and in spruce around 1000 r. Low doses between 75 and a few hundred r-units appear to stimulate germination or growth. The two conifer species are consequently rather sensitive to X-irradiation, more so than most agricultural plants examined (GUSTAFSSON, 1944). The alder species, on the other hand, stands

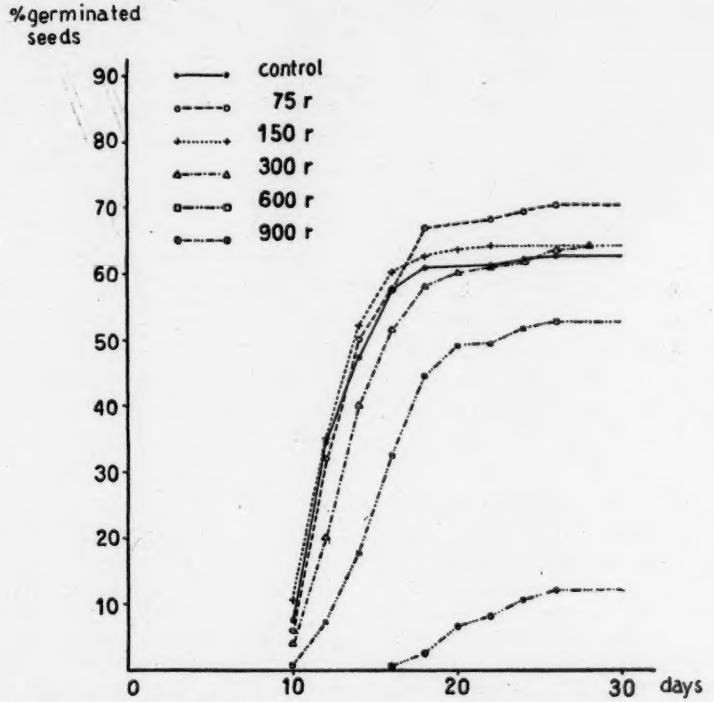


Fig. 8. Seed germination in Norway spruce (*Picea Abies*) after increasing X-ray dose. (Series 2, p. 465.)

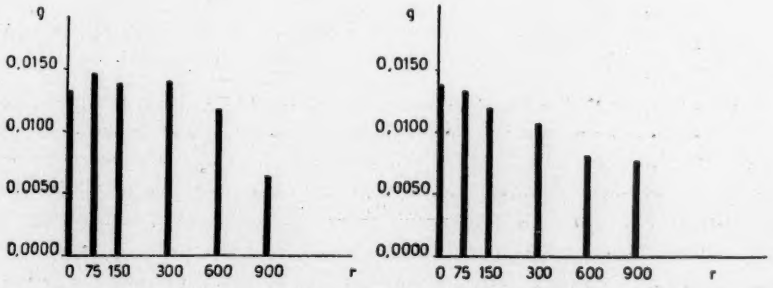


Fig. 9. Average seedling weight in Scot's pine (left) and Norway spruce (right) after increasing X-ray dose. (Series 2, p. 465, laboratory analysis.)

doses up to the level characteristic of the pea species, of *Helianthus* and *Carthamus*.

The dose produced by the exposure with X-ray photography has, owing to technical difficulties, not been definitely measured so far. There is no doubt that it lies below the lethalizing doses of 600—1000 r in pine and spruce. According to rough estimates it is *circa* one tenth or less of this dose. It is therefore negligible for practical purposes, even if a full germinability and growth is to be kept. Still it may induce a very low percentage of lethal recessives and chromosome rearrangements. Two tasks are therefore close at hand: (1) to measure the dose involved in X-ray photography accurately, for instance by means of the chemical dosimetry used by EHRENBERG and NYBOM (1952), and (2) to decrease this dose by technical improvements of the X-ray photography itself.

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A GENETICAL STUDY IN THE MODE OF SEGREGATION IN HEXAPLOID PHLEUM PRATENSE

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FOR a long time there has been discussion on the origin of the hexaploid *Phleum pratense* and the mode of polyploidy it holds (GREGOR and SANSOME, 1930; GREGOR, 1945—46; MÜNTZING and PRAKKEN, 1940; NORDENSKIÖLD, 1945, 1949; MYERS, 1944; and STEBBINS, 1950, p. 333). One way in which this question may be studied is by investigating the mode of segregation taking place in this hexaploid species. Such a study can give an answer to what mode of pairing the chromosomes of the plants undergo in meiosis, if the pairing be of a di- or polysomic type. To get a closer knowledge of these properties in this particular species, a study has been made on the occurrence of white seedlings in progenies obtained after isolation of individual plants.

For this investigation the »Omnia» strain of timothy of Svalöf was used. The reasons for choosing this strain were two. Firstly, the strain is known to be rather self-fertile, a property which the plants used for this investigation must possess, in order to give a sufficient amount of seeds for the study; secondly, the strain originates from one single plant. Only relatively few generations have passed since the harvest of this original plant (JULÉN, 1947). Thus the single plants of the strain are relatively closely related to each other.

The investigation was begun in 1950 by making isolations of 21 normal vigorous plants of timothy of the »Omnia» strain. The isolations of the first year (1950) gave rather few seeds from only 17 of the 21 plants. In order to obtain more seeds, the isolations of the same plants were repeated in 1951 and 1952. The seeds yielded after isolation were germinated in petri dishes, 100 seeds in each dish, green and white seedlings as well as non-germinated seeds being counted (see Table 1).

During 1951 and 1952, a thorough examination of different properties of the 21 plants under investigation was performed. Seed setting

TABLE 1. *Observed ratios of green and white seedlings for the investigated plants of P. pratense, fertility properties of the parental plants, and distribution of genotypic groups of the I_1 plants.*

Plant No.	Total number of seedlings 1950—1952		Segreg. found: white/green	Hypothetical segregations			Germination per cent	Seeds per cm of ear, isolat.	Seeds per cm of ear, open poll.	Per cent good poll.	Number of I_1 plants belonging to indicated genotypic groups				I_1 plants classif.	I_1 plants invest.
	Green	White		Segreg.	Exp. whites	P-values					Non-segreg.	Triplex	Duplex	Simplex		
Omnia 1	2366	18	1 : 131	1 : 99,4 1 : 132,7	23,80 17,83	0,3 —0,2 0,98—0,95	98	4	42	95	2	2	1		5	9
2	1526	14	1 : 109	1 : 99,4 1 : 132,7	15,34 11,52	0,8 —0,7 0,5 —0,3	95	4	43	93	1	1			2	13
3	512	11	1 : 47				90	1	45	85					0	17
4	5634	22	1 : 256	1 : 199,3 1 : 399	28,24 14,14	0,3 —0,2 0,95—0,92	98	36	—	95	1	5	3		9	9
5	881	0					98	3	50	81					0	17
6	547	1	(1 : 547)				96	2	18	89					0	18
7	773	1	(1 : 773)				95	2	40	95	1		2		3	19
8	370	17	1 : 22	1 : 22,6 1 : 24	16,40 15,48	0,9 —0,8 0,7 —0,5	90	1	30	91		1	4	1	6	10
9	328	6	1 : 55				95	1	53	94		1	1		2	10
10	833	1	(1 : 833)				95	2	52	92	1			1	2	5
11	3716	17	1 : 219	1 : 199,3	18,64	0,8 —0,7	95	15	35	92	1				1	8
12	1475	1	(1 : 1475)				98	7	50	97	1	2	1		4	9
13	3758	146	1 : 26	1 : 22,6 1 : 24	166,28 156,58	0,2 —0,1 0,5 —0,3	96	16	42	97			4	2	6	15
14	3327	11	1 : 302	1 : 199,3 1 : 399	16,67 8,35	0,2 —0,1 0,5 —0,3	97	9	39	95	1	3	3		7	15
15	2923	9	1 : 325	1 : 199,3 1 : 399	14,64 7,33	0,2 —0,1 0,7 —0,5	98	34	61	97	2	6	8		16	18
16	432	5	1 : 86	1 : 99,4	4,35	0,95—0,90	88	0,3	36	73			1		1	14
17	384	1	(1 : 384)				96	1	44	94	1	1	3		5	17
18	1329	61	1 : 22	1 : 22,6 1 : 24	58,80 55,38	0,8 —0,7 0,5 —0,3	95	4	30	96	2	3	1	2	8	26
19	223	0					91	0,5	—	89	—	—	—	—	—	—
20	667	0					96	2	52	96	4	1	2		7	21
21	101	1	(1 : 101)				98	0,5	17	89	2	2	1		5	30

ability after isolation and open pollination was examined, as well as pollen fertility (Table 1). No notable observations were made in this study. One plant (No. 16) had a lowered male fertility and two individuals (Nos. 6 and 21) a rather low female fertility. In 1952 meiosis of the plants was investigated in order to get a knowledge of the frequency of multivalents. As expected, the observed multivalents appeared very rarely. Only a few quadrivalents were met with in each plant among about 100 cells studied and never more than one quadrivalent in a cell. This result is in accordance with earlier investigations in European material of *P. pratense* (MÜNTZING and PRAKKEN, 1940; NORDENSKIÖLD, 1945). Moreover, in some of the plants univalents were found during meiosis. A certain number of univalents was previously observed in the material of *P. pratense* studied by MYERS (1944).

To obtain an understanding of the mode of segregation which the different ratios between white and green seedlings may represent, it has been necessary to calculate the ratios of di- and polysomic types of segregation which theoretically one might expect to obtain in a hexaploid. For such calculations it is necessary to postulate the most likely behaviour of the genes for albino seedlings in *P. pratense*. Genes for albino seedlings seem to behave in a rather similar way in the different species previously investigated. Detailed studies of the occurrence of such albino seedlings have been made in several grasses, predominantly in cereals such as barley, different species of wheat and oats as well as in maize. Barley is the most thoroughly investigated species from this point of view. It is diploid and self-fertilizing, which makes it a very suitable subject for such studies. From the investigations of that species, it is known that genes inducing albino seedlings occur in different loci in the species. Some of them segregate independently (NILSSON-EHLE, 1922) but linkage between them is also known (HALLQVIST, 1926). When two genes for albino seedlings have an independent segregation, the second generation gives the ratio of 7 white to 9 green. This relation shows that the recessive homozygotes of both the genes are white, and that the phenotypes of the two homozygotes in these cases are impossible to distinguish from each other.

When we come to polyploids, *e. g.*, hexaploid wheat or hexaploid oats, we get another segregation pattern on account of the polyploidy. It is a well known fact that the mutation ratio in such polyploids is much lower than that for diploids. In accordance with this fact, albino seedlings of wheat and oats are very scarce in nature. STADLER (1929) has given an explanation of this difference in frequency of chlorophyll

TABLE 1. *Observed ratios of green and white seedlings for the investi*

mutations of diploids and polyploids. In his theory, he explains the lower frequency of mutations in polyploids by assuming reduplication of genes in corresponding chromosomes of the last-mentioned species. As a result of this reduplication of genes, the ratios of recessives and dominants of hexaploids, *e. g.*, oats and wheat, turn out to give polymeric segregations as 1 : 63, 1 : 15, or 1 : 3, depending on how many of the reduplicated genes are heterozygous. These ratios have been found for genes of different chlorophyll deficiencies in oats (FRÖIER, 1946), showing that this species has a disomic segregation with three homologous polymeric factors probably caused by reduplication of genes in corresponding chromosomes. A comparable segregation ratio for an autohexaploid with hexasomic inheritance would be 1 : 399, 1 : 24, or 1 : 3, if we calculate with random assortment of chromosomes only. Such a calculation is permissible for the strain of timothy studied here, as the multivalents in meiosis almost failed. On the other hand, multivalents are formed, so that random assortment of chromatids can occur and must not be wholly neglected, although it cannot change the segregation ratios in any appreciable way.

If we look at Table 1, we find that almost every investigated plant contains recessive genes for white seedlings. The only one that has not been proved to contain such genes (No. 19) has been rather self-sterile, so that the number of seedlings investigated have been too small to really prove anything, and no progeny test has been taken from that plant. We know from previous investigations that the thoroughly investigated barley has several different genes for albino seedlings, which phenotypically cannot be separated (NILSSON-EHLE, 1922; HALLQVIST, 1926). Thus, it is very likely that this is also the case with timothy. For that reason, we must presume that the genes for the white seedlings in the present material are not all located in equivalent loci of corresponding chromosomes. Most likely, many of the investigated plants contain such genes in more than one locus and, thus, usually in more than one of their chromosome »sextettes». For that reason, tables have been calculated giving the different segregation ratios for di- and polysomic inheritance with a different number of recessive genes in equivalent loci of one or two of the chromosome »sextettes» of a hexaploid (Tables 2, 3, and 4).

Table 2 gives the disomic segregation of a hexaploid, Table 4 the hexasomic, and Table 3 a segregation where four of the six genomes are presumed to have a tetrasomic segregation and the remaining two a disomic one. The first column of the tables gives the genetic con-

TABLE 2. *Calculated ratios of recessives and dominants for a hexaploid with disomic segregation and reduplicated genes in corresponding chromosomes. Calculation made for one and two factor sets.*

Parental plant			Number of I_1 plants belonging to indicated genotypic groups			
Genotype	Genotypic group	Segregation ratio	Non-segregating	Trihybrid or 3rd	Dihybrid or 2nd	Monohybrid or 1st
Aabbcc	monohybrid or 1st	1 : 3	33,3	—	—	66,7
AaBbcc	dihybrid or 2nd	1 : 15	46,6	—	26,7	26,7
AaBbCc	trihybrid or 3rd	1 : 63	58,7	12,7	19,1	9.5
Aabbcc Xxyyzz	1st	1 : 1,3	11,1	—	—	88,9
Aabbcc XxYyzz	1st	1 : 2,4	15,5	—	8,9	75,6
Aabbcc XxYyZz	1st	1 : 2,8	19,6	4,2	6,3	69,9
AaBbcc XxYyzz	2nd	1 : 7,3	21,7	—	32,0	46,3
AaBbcc XxYyZz	2nd	1 : 12,0	27,4	5,9	33,0	33,7
AaBbCc XxYyZz	3rd	1 : 31,3	34,5	16,5	30,9	18,1

stitution of the parental plant (the factor set of the first chromosome »sextette» has been denoted by the letters *a*, *b* or *c*, the one of the second by *x*, *y* or *z*, and so on) and the third one the segregation ratio expected between recessives and dominants. The second column gives the genotypic group to which the parental plant has been referred. For practical purposes it has been found necessary to pool plants of different genetical constitution having segregation ratios which statistically cannot be distinguished from each other. For that reason, the plants have been referred to the different groups given in the second column of the tables. Plants of those groups are determined by the highest number of recessive genes present in a certain locus of any of its chromosome »sextettes». Thus, the groups in Table 4 are: Simplex or 1st group, indicating that a locus in one of the chromosome »sextettes» contains at least five recessives; duplex or 2nd group, indicating that at least one of the chromosome »sextettes» holds at least four recessives in one locus; the triplex or 3rd group indicates in the same way that no locus of the chromosome »sextettes» has more than three recessives; and the quadruplex or 4th group indicates that one of the chromosome »sextettes» contains at least two recessives in one locus. Corresponding genotypic groups have been denoted in the same column of Tables 2 and 3.

For polysomic types of segregation there is, however, still another phenomenon to take into consideration, the result of which is

TABLE 3. *Calculated ratios of recessives and dominants for a hexaploid with tetra-disomic segregation, i. e. a hexaploid with four of its six genomes giving tetrasomic segregation and the remaining two with reduplicated genes in corresponding chromosomes showing disomic segregation. Calculation made for one and two factor sets.*

Parental plant			Number of I_1 plants belonging to indicated genotypic group			
Genotype	Genotypic group	Segregation ratio	Non-segregating	3 a	2 a or 2 b	1 a or 1 b
a_4Bb	1 a	1 : 3	33,3	—	—	66,7
A_1a_3bb	1 b	1 : 3	—	—	33,3	66,7
A_1a_3Bb	2 a	1 : 15	26,7	13,3	33,3	26,7
A_2a_2bb	2 b	1 : 35	25,7	—	51,4	22,9
A_2a_2Bb	3 a	1 : 143	44,0	25,2	23,8	7,0
A_3a_1bb	3 b	not segreg.	75	—	25	—
A_3a_1Bb	4 a	not segreg.	81,3	12,5	6,2	—
$a_4Bb\ x_4Yy$	1 a	1 : 1,3	11,1	—	—	88,9
$a_4Bb\ X_1x_3yy$	1 a	1 : 1,3	—	—	11,1	88,9
$a_4Bb\ X_1x_3Yy$	1 a	1 : 2,4	8,9	4,4	11,1	75,6
$a_4Bb\ X_2x_2yy$	1 a	1 : 2,7	8,6	—	17,1	74,3
$a_4Bb\ X_2x_2Yy$	1 a	1 : 2,9	14,7	8,4	7,9	69,0
$A_1a_3bb\ X_1x_3yy$	1 b	1 : 1,3	—	—	11,1	88,9
$A_1a_3bb\ X_1x_3Yy$	1 b	1 : 2,4	—	—	24,4	75,6
$A_1a_3bb\ X_2x_2yy$	1 b	1 : 2,7	—	—	25,7	74,3
$A_1a_3bb\ X_2x_2Yy$	1 b	1 : 2,9	—	—	31,0	69,0
$A_1a_3Bb\ X_1x_3Yy$	2 a	1 : 7,3	7,1	8,9	37,8	46,2
$A_1a_3Bb\ X_2x_2yy$	2 a	1 : 10,3	6,9	3,4	46,3	43,4
$A_1a_3Bb\ X_2x_2Yy$	2 a	1 : 13,5	11,7	16,0	40,5	31,8
$A_1a_3Bb\ X_3x_1yy$	2 a	1 : 15	20,0	10,0	43,3	26,7
$A_1a_3Bb\ X_3x_1Yy$	2 a	1 : 15	21,7	15,8	35,8	26,7
$A_2a_2bb\ X_2x_2yy$	2 b	1 : 17,3	6,6	—	52,9	40,5
$A_2a_2bb\ X_2x_2Yy$	2 b	1 : 28,0	11,3	6,5	54,0	28,2
$A_2a_2bb\ X_3x_1yy$	2 b	1 : 35	19,3	—	57,8	22,9
$A_2a_2bb\ X_3x_1Yy$	2 b	1 : 35	20,9	3,2	53,0	22,9
$A_2a_2Bb\ X_2x_2Yy$	3 a	1 : 71,3	19,4	28,5	38,6	13,5
$A_2a_2Bb\ X_3x_1yy$	3 a	1 : 143	33,0	18,9	41,1	7,0
$A_2a_2Bb\ X_3x_1Yy$	3 a	1 : 143	35,8	29,1	28,1	7,0
$A_3a_1bb\ X_3x_1yy$	3 b	not segreg.	56,3	—	43,7	—
$A_3a_1bb\ X_3x_1Yy$	3 b	not segreg.	60,9	9,4	29,7	—
$A_3a_1Bb\ X_3x_1Yy$	4 a	not segreg.	66,0	21.9	12,1	—

not shown in the tables. This is the random assortment of chromatids manifested only when multivalents are formed in meiosis. As multivalents appear very rarely in this strain of timothy, random assortment

TABLE 4. *Calculated ratios of recessives and dominants for a hexaploid with hexasomic segregation. Calculation made for one to four factor sets.*

Parental plant			Number of I_1 plants belonging to indicated genotypic groups			
Genotype	Genotypic group	Segregation ratio	Non-segregating	Triplex or 3rd	Duplex or 2nd	Simplex or 1st
A_1a_5	simplex or 1st	1 : 3	—	—	33,3	66,7
A_2a_4	duplex or 2nd	1 : 24	4,2	25,0	45,8	25,0
A_3a_3	triplex or 3rd	1 : 399	29,6	41,1	24,8	4,5
A_4a_2	quadruplex or 4th	not segreg.	72,0	24,0	4,0	—
$A_1a_5\ X_1x_5$	1st	1 : 1,28	—	—	11,1	88,9
$A_1a_5\ X_2x_4$	1st	1 : 2,57	—	—	25,0	75,0
$A_1a_5\ X_3x_3$	1st	1 : 2,97	—	—	31,8	68,2
$A_2a_4\ X_2x_4$	2nd	1 : 11,8	0,2	8,3	47,7	43,8
$A_2a_4\ X_3x_3$	2nd	1 : 22,6	1,2	19,4	51,0	28,4
$A_2a_4\ X_4x_2$	2nd	1 : 24	3,0	25,0	47,0	25,0
$A_3a_3\ X_3x_3$	3rd	1 : 199,3	8,8	41,2	41,2	8,8
$A_3a_3\ X_4x_2$	3rd	1 : 399	21,3	46,6	27,6	4,5
$A_4a_2\ X_4x_2$	4th	not segreg.	51,9	40,3	7,8	—
$A_3a_3\ X_3x_3\ P_3p_3$	3rd	1 : 132,7	2,8	32,7	51,8	12,9
$A_3a_3\ X_3x_3\ P_4p_2$	3rd	1 : 199,3	6,3	41,7	43,2	8,8
$A_3a_3\ X_3x_3\ P_3p_3\ S_3s_3$	3rd	1 : 99,4	0,8	24,2	58,2	16,8

of the chromatids will not change the segregation ratios to any appreciable extent. The only result of that phenomenon visible in the segregation ratios is the fact that we actually can obtain homozygote recessives from plants of quadruplex constitution by mere segregation. Thus, this will cause the occurrence of single recessive seedlings in the progeny of such plants, or in other words, we can receive recessives from quadruplex plants in a very low frequency.

The last column of Tables 2, 3, and 4 gives the ratio of the different genotypic groups of the I_1 plants. These plants of the first isolated generation have been classified according to the segregation ratios of the seedlings obtained after isolation from those plants. The white homozygotes have thus been left out of consideration, as they are non-vital plants giving no progeny. In Table 4 the heterozygous I_1 plants have, thus, been classified into four groups, simplex, duplex, triplex, and a »non-segregating» group to which all not segregating plants, the quadruplex and quintuplex included, have been referred.

The segregation ratio of many of the investigated plants may, of

course, be much more intricate than has been shown in the tables. Different genes for albino seedlings might be situated in the same chromosome »sextette» and thus linked. But this complication will give segregation ratios lying between the ones calculated in the tables, and will thus not change the genotypic group of the plants. Other complications, such as sterility causing deficiencies in certain kinds of segregates are, moreover, always to be expected in some individuals, since the plants investigated have been taken at random out of a more or less natural, cross-pollinating population.

In Table 1, different columns are found which correspond to those of the calculated tables. Thus, the first part of the table gives the segregation ratios and other properties of the mother plant. The second part of the table shows the data concerning the progeny. The last column gives the number of I_1 plants investigated. The majority of the I_1 plants have been planted in 1952 and harvested during only one year (1952), a few of the progenies (Nos. 4, 8, 13, and 15) were, however, planted already in 1951 and thus harvested twice. Among the I_1 plants investigated many were completely sterile and others have yielded only a few seeds after isolation. On this account the classified plants, given in the preceding column, are much less numerous than the investigated ones. The I_1 plants giving 100 seedlings have been classified even though it is obvious that the »non-segregating» plants will become excessive at the expense of the triplex ones. To classify the plants of the simplex or duplex group, less than 100 seedlings usually have been necessary. As those plants containing a higher number of recessive genes usually have given less seedlings than the former ones, it has been considered most correct to classify them even when they have yielded less seedlings than 100, provided that a sufficient number of seedlings have been produced for a statistically significant separation of the groups.

The comparison between the experimental ratios obtained (Table 1) and the calculated ones (Tables 2, 3, and 4) are given below, together with a more thorough analysis of each mother plant and its progeny.

At first we will analyse the families which have given the highest frequency of recessives in the first isolated generation. These are Nos. 8, 13, and 18 (Table 1). They seem to show the highest frequency of white seedlings which normally is found in this strain of timothy. The segregation ratios of all three plants fit well with 1 : 24 or 1 : 22,6, *i. e.* the duplex group of the hexasomic segregation (Table 4), when the ratios are compared and tested with the χ^2 test (Table 1). The ratios

of plants Nos. 13 and 18 did not fit significantly with 1 : 15 ($P < 0{,}001$ for No. 13 and $P < 0{,}01$ for No. 18). Nor did the ratios for those plants fit satisfactorily with 1 : 35 ($P < 0{,}001$). The only tetra-disomic segregation ratio from which the ratios of those plants might be derived is 1 : 28,0 (Table 3). Good significance for this ratio is obtained for plant No. 13 where P falls between 0,5 and 0,3, whereas plant No. 18 lies on the border-line of significance with $P = 0{,}05$. Plant No. 8 has not yielded a sufficient amount of seedlings to give a statistically significant deviation from any of those three tetra-disomic ratios discussed.

When the genotypic groups of the I_1 plants are tested against corresponding calculated distributions, the families Nos. 8 and 13 give a good significance with both the postulated hexasomic segregations (Tables 5 and 6). No. 18 is, however, on the border-line of statistical significance ($P = 0{,}06$). As the I_1 plants tested are not more than 6 to 8, they have been classified into only two classes by comparison with the theoretically expected values: one class containing the plants which have not given any recessive segregates together with those of the triplex or 3rd group (»non-segreg.+3«) and a second class comprising the plants of the simplex or 1st group together with the ones of the duplex or 2nd group (»1+2«). On account of the low number of I_1 plants tested the probabilities of the genotypic groups of the I_1 plants have been calculated from the binominal distribution (the probabilities for the postulated ratios of the parental plants are in the tables indicated after the plant number for comparison).

If distribution of the genotypic groups of the I_1 plants are tested against the corresponding distribution of the postulated tetra-disomic

TABLE 5. *Comparison between observed and expected ratios of the genotypic groups of I_1 plants originating from mother plants with the postulated genetical constitution of A_2a_4.*

	Plant No. 8 ($0{,}7 > P > 0{,}5$)		Plant No. 13 ($0{,}5 > P > 0{,}3$)		Plant No. 18 ($0{,}5 > P > 0{,}3$)	
Genotypic groups of I_1 pl.	»Non-segreg. + 3«	»1 + 2«	»Non-segreg. + 3«	»1 + 2«	»Non-segreg. + 3«	»1 + 2«
I_1 pl. observ.	1	5	0	6	5	3
I_1 pl. expect.	1,75	4,25	1,75	4,25	2,34	5,66
	$P = 0{,}68$		$P = 0{,}19$		$P = 0{,}06$	

TABLE 6. *Comparison between observed and expected ratios of the genotypic groups of I_1 plants originating from mother plants with the postulated genetical constitution of $A_2a_4X_3x_3$.*

	Plant No. 8 ($0{,}9 > P > 0{,}8$)		Plant No. 13 ($0{,}3 > P > 0{,}1$	
Genotypic groups of I_1 pl.	»Non-segreg. + 3»	»1 + 2»	»Non-segreg. + 3»	»1 + 2»
I_1 pl. observ.	1	5	0	6
I_1 pl. expect.	1,24	4,76	1,24	4,76
	P = 1		P = 0,36	

genotype ($A_2a_2bb\ X_2x_2Yy$), significance is obtained for plants Nos. 8 ($P > 0{,}9$) and 13 ($P = 0{,}38$). Plant No. 18 gives, however, no significance in the test ($P = 0{,}006$).

From these tests of two generations, it seems as if the families Nos. 8 and 13 can be derived from either hexasomic or tetra-disomic mode of segregation, plant No. 18, on the other hand, might be derived from the hexasomic genotype A_2a_4, even though it has given an excess of »non-segregating» and triplex I_1 plants.

Secondly, the families will be analysed, the parental plants of which have given segregations derived from some kind of triplex constitution. To this group of families are counted Nos. 1, 2, 4, 11, 14, and 15. Plants which have yielded only one single recessive (Nos. 6, 7, 10, 17, and 21) are left out of consideration, even if it is most likely that they belong to this group of plants. The six first-mentioned plants have throughout been rather self-fertile. They have given segregation ratios which might be derived from different ratios of the triplex or 3rd hexasomic group, such as 1 : 399, 1 : 199,3 1 : 132,7, or 1 : 99,4 (Tables 1 and 4). The tetra-disomic segregation ratio 1 : 143 gives, moreover, significance for the seedlings obtained from Nos. 1, 2, and 11. The progenies of these three plants are very self-sterile, so it is impossible to obtain any separating evidence from those I_1 plants to what kind of genotype the mother plant holds. None of the six plants referred to this triplex group have yielded segregation ratios, which give significance for 1 : 63. There is, however, still another disomic type of polymeric segregation which these plants might be derived from. That is from the segregation ratio 1:255, obtained when four polymeric factors are postulated to segregate independently. Plants Nos. 4, 11, 14, and 15

give significance, when tested against this ratio. The distribution of the genotypes of the I_1 plants does not, however, give any significance for the families Nos. 4, 14, and 15, which are the ones sufficiently fertile to allow a test. When four polymeric factors are postulated, »non-segregating» I_1 plants amount to 68,6 per cent of the progeny. When this relation is tested against the number of »non-segregating» plants, observed in these families (*cf.* Table 1), no significance is obtained ($P < 0{,}001$ for plants Nos. 4 and 15; $P = 0{,}005$ for plant No. 14). There is, thus, no reason to believe that four polymeric factors with disomic segregation would control the inheritance of albino seedlings in these plants.

TABLE 7. *Comparison between observed and expected ratios of the genotypic groups of I_1 plants originating from mother plants with the postulated genetical constitution of A_3a_3.*

	Plant No. 4 ($0{,}05 > P > 0{,}02$)		Plant No. 14 ($0{,}5 > P > 0{,}3$)		Plant No. 15 ($0{,}7 > P > 0{,}5$)	
Genotypic groups of I_1 pl.	»Non-segreg. + 3»	»1 + 2»	»Non-segreg. + 3»	»1 + 2»	»Non-segreg. + 3»	»1 + 2»
I_1 pl. observ.	6	3	4	3	8	8
I_1 pl. expect.	6,36	2,64	4,95	2,05	11,31	4,69
	$P = 1$		$P = 0{,}68$		$P = 0{,}11$	

TABLE 8. *Comparison between observed and expected ratios of the genotypic groups of I_1 plants originating from mother plants with the postulated genetical constitution of $A_3a_3X_3x_3$.*

	Plant No. 4 ($0{,}3 > P > 0{,}2$)		Plant No. 14 ($0{,}2 > P > 0{,}1$)		Plant No. 15 ($0{,}2 > P > 0{,}1$)	
Genotypic groups of I_1 pl.	»Non-segreg. + 3»	»1 + 2»	»Non-segreg. + 3»	»1 + 2»	»Non-segreg. + 3»	»1 + 2»
I_1 pl. observ.	6	3	4	3	8	8
I_1 pl. expect.	4,50	4,50	3,50	3,50	8,00	8,00
	$P = 0{,}51$		$P = 1$		$P = 1$	

Among the plants referred to the triplex group there are only three which have given a sufficient number of seeds in the second generation to allow any statistical test of the latter generation. Those plants are Nos. 4, 14, and 15. The distribution of the genotypic groups of the I_1 plants originating from Nos. 4, 14, and 15 give, when tested against expected ratios, significance with the two genotypes A_3a_3 and $A_3a_3\,X_3x_3$, as shown in Tables 7 and 8.

Comparing the results of the statistical tests of the first and second generations of those plants in question, it seems as plants Nos. 14 and 15 might be derived from either postulated hexasomic genotype, whereas plant No. 4 probably is of the complex hexasomic type. The disomic or tetra-disomic mode of segregation cannot be statistically proved for any of those plants.

At last we will study the plants, which in the first isolated generation have not given any white seedlings at all, *viz.* Nos. 5 and 20, together with plant No. 12, which has given only one recessive among 1475 green seedlings. As all these plants have yielded white seedlings in the second generation, they ought to be classified as belonging to the quadruplex or in some cases the triplex group. Plant No. 12 ought to belong to the first-mentioned group. The single white seedling of that plant might have segregated out by chromatid assortment from a plant of quadruplex constitution. Single quadrivalents were observed in meiosis of that plant as in many of the other ones studied, and thus such a segregation is possible in isolated cases. Among the I_1 plants of family 12, no one of the simplex group has been observed. The genotypic groups of the tested I_1 plants of this family were as follows: one of the duplex group, two of the triplex, and one »non-segregating». The quadruplex constitution of the mother plant thus corresponds with the genotypic groups of the progeny plants (*cf.* Table 4). The mother plants of the other two families (Nos. 5 and 20) have only given green seedlings, but the number of seedlings is not very high, only 881 and 667 seedlings, respectively, having been obtained. The progeny of No. 5 was very self-sterile and the ears usually small and abnormal, so that no I_1 plants with more than 43 seedlings were obtained. Three plants, however, have given single white seedlings. Plant No. 20 yielded a more fertile progeny and the distribution of the genotypic groups of the I_1 plants tested against the theoretically calculated ratios of some of the most likely genotypes is recorded in Table 9.

Table 9 shows that when one quadruplex factor set is assumed to be involved, no statistical significance is obtained, but when two

TABLE 9. *Comparison between observed and expected ratios of the genotypic groups of the I_1 plants originating from plant No. 20.*

	Hypothet. genotype of mother plant A_4a_2		Hypothet. genotype of mother plant $A_4a_2\ X_4x_2$		Hypothet. genotype of mother plant A_3a_3	
Genotypic groups of I_1 pl.	»Non-segreg. + 3»	»1 + 2»	»Non-segreg. + 3»	»1 + 2»	»Non-segreg. + 3»	»1 + 2»
I_1 pl. observ.	5	2	5	2	5	2
I_1 pl. expect.	6,72	0,28	6,45	0,55	4,95	2,05
	P = 0,03		P = 0,10		P = 1	

quadruplex factor sets are postulated, the distribution of the genotypic groups of the I_1 plants might be derived from the expected values. When a triplex constitution of the mother plants is assumed, the distribution of the I_1 plants fit very well with the theoretically expected distribution. Among the ratios derived from tetra-disomic segregations (Table 3) there are, moreover, some plausible genotypes ($A_3a_1bb\ X_3x_1Yy$ or $A_3a_1Bb\ X_3x_1Yy$) from which this family No. 20 might be derived. No disomic genotype is, however, able to explain the segregation ratios of this family.

Going back to Table 1, there are still three plants which have not been discussed. Those are Nos. 3, 9, and 16. They have yielded segregation ratios which are somewhat difficult to interpret. No. 16 has given 432 green to 5 white, which might be derived from 1 : 99,4 (Table 1), No. 9 has yielded 328 green to 6 white, which ratio theoretically might be derived from a still more complex triplex genotype. The same is the case with No. 3, giving 512 green to 11 white. All three plants are rather self-sterile, for which reason it is likely that they show disturbances of their segregation ratios. Thus, it is very plausible that at least plants Nos. 3 and 9 could be of some duplex constitution with a strong deficit of recessives. Single individuals of this type have been met with also in the progeny of the other plants investigated. So have two I_1 plants of No. 4 given 5 : 270 and 5 : 259, respectively, and one I_1 plant of No. 17 yielded 4 : 219. All these plants have been rather self-sterile. As there seems to be a definite tendency for self-sterile duplex plants to have a deficit of recessives, these plants in question have been referred to the duplex group. On this same account, it is very likely that plants Nos. 3 and 9 also would be of such a duplex

type, the segregation ratio of which has been disturbed by the self-sterility or other irregularities of the plants. The progenies of these plants are so sterile that no evidence can be obtained by studying the I_1 plants. From a statistical point of view, all these plants might be derived from the ratio 1 : 63 or some complex tetra-disomic segregation. But single progeny plants and sister individuals of the plants have given segregation ratios which hardly can be explained by such a mode of segregation. And all of them do belong to more or less sterile plants, among which disturbances of the segregation ratio are to be expected; for that reason a definite conclusion concerning their mode of segregation is very difficult to draw.

Earlier investigations on the inheritance of genes for albino seedlings in hexaploid *Phleum pratense* have been made by WEXELSEN (1941) and MYERS (1944). In the first-mentioned paper, WEXELSEN calculated with three homomeric factors and disomic segregation. He found segregation ratios which he derived from 1 : 3, 1 : 15, and 1 : 63. The green and white seedlings he calculated with to get the ratio 1 : 63 were, however, obtained from summation of the progeny of 24 different plants, yielding between 593 and 36 seedlings each, the majority of the plants giving less than 150 seedlings. About half the number of plants have given only one single recessive. As the plants giving no recessives were left out of consideration, he received an average number of recessives among those plants much larger than would have been obtained if a higher number of seedlings had been tested from each plant. In spite of this fact the total number of albinos observed (47) were only about half that of the expected ones (83,03), when compared with the segregation ratio 1 : 63. On this account, the figures given by WEXELSEN cannot be considered as any proof of a disomic inheritance in hexaploid *Phleum pratense*.

MYERS (1944) has made an intense study of the inheritance of white seedlings in hexaploid *Phleum pratense*. He studied seedlings from both first and second generations of the investigated plants, and did not find any support for disomic inheritance in the plants investigated. The derivation from a tetra-disomic mode of inheritance *versus* that from a hexasomic one was impossible to distinguish with the limited material he had available. There were, however, two families, the segregation ratios of which did not fit into the calculated hexasomic schedule. However, by the calculation of this schedule, segregations with only one factor set had been taken into consideration. If genes for albino seedlings are postulated in two or three of the chro-

mosome »sextettes», ratios will be obtained which give significance for a hexasomic segregation in both these two families. The two families in question were denoted Ti 11 (8) and Ti 16 (5). The parental plant of Ti 11 (8) had given 557 green seedlings after isolation. In the I_1 generation 14 plants did not segregate and 9 did segregate. The 557 green seedlings of the parental plant might be derived from 1 : 143, a segregation ratio which is on the border-line of statistical significance ($0{,}05 > P > 0{,}02$). It is more likely to be derived from some hexasomic segregation ratio as 1 : 399 ($0{,}3 > P > 0{,}2$). Among the segregating I_1 plants there was, however, one plant yielding 127 green to 14 white. This ratio can hardly be derived from 1:24, which would be the nearest segregation ratio possible, for a plant originating from the genotype A_3a_3. If the postulated genotype of the parental plant is given as $A_3a_3\ X_4x_2$, the plant would have the same segregation ratio in the first generation, 1 : 399. In the second generation, however, plants of the genotype $A_2a_4\ X_2x_4$ are expected. The segregation ratio of such a plant would be 1 : 11,8 (Table 4), a ratio from which 14 : 127 might be derived ($0{,}5 > P > 0{,}3$). The expected values for »non-segregating» plants in the second generation would with this premise be 15,6, and the corresponding value for the »segregating» is 7,4. Compared with the 14 »non-segregating» and 9 »segregating» plants observed, a probability between 0,5 and 0,3 is obtained. [The calculated triplex I_1 plants have been pooled with the »non-segregating» ones, which seems to be most correct with the low number of seedlings (average 79,4) tested.] Thus, family Ti 11 (8) seems to give statistical significance for the hexasomic mode of segregation.

So does also the family denoted Ti 16 (5). The parental plant of the family gave 575 green seedlings to 4 white, a segregation ratio which can be derived from 1 : 143, 1 : 120, 1 : 86,1 or 1 : 63 (MYERS, *l. c.*). But it might also be derived from 1 : 132,7 or 1 : 199,3. Among the I_1 plants, 5 were »non-segregating» whereas 18 were »segregating». None of the segregating plants showed a ratio approximating 1 : 3. The distribution of the genotypes of the I_1 plants did not, however, give statistical significance when compared against expected ratios if any of the tetra-disomic or disomic genotypes in question were presumed. Only from the genotype A_3a_3 with chromatid assortment the derivation is possible (MYERS, *l. c.*). A likely genotype of the parental plant, which also gives statistical significance for both generations tested, would, moreover, be $A_3a_3X_3x_3P_3p_3$ with a segregation ratio for the parental plant of 1 : 132,7 (Table 4). The segregation ratio of the mother plant

Ti 16 (5) was 4 : 575, a ratio which can be derived from 1 : 132,7 ($0{,}9 > P > 0{,}8$). With this premise the expected values for »non-segregating» and »segregating» I_1 plants would be 8,1 and 14,9, respectively, to compare with the observed ones, 5 »non-segregating» to 18 »segregating» ($0{,}2 > P > 0{,}1$), if the triplex I_1 plants are added to the »non-segregating». Out of the segregating plants obtained, only 2,97 are expected to give a simplex segregation ratio of about 1 : 3. That none have appeared in the material studied might be expected to occur by chance only ($0{,}1 > P > 0{,}05$). As far as the published figures show (MYERS, 1944), the segregation ratios of this family can thus be explained by postulating a hexasomic segregation, with or without the assumption of chromatid assortment.

DISCUSSION.

The present investigation of the frequency of white seedlings in hexaploid *Phleum pratense* has been made in order to illustrate what mode of segregation the genes of this species respond to. The most striking result of this study seems, however, to be the demonstration of the very high frequency of genes for white seedlings which are concealed in this strain of timothy. Everyone of the 20 plants, which have been tested in two consecutive generations, contains genes for white seedlings. This very high frequency of white seedlings might be typical for the »Omnia» strain of timothy only. Previously, however, a rather high frequency of chlorophyll deficient seedlings has been observed in the progeny of isolated lines of timothy (CLARKE, 1927; WEXELSEN, 1941). Still, not more than 30 to 40 per cent of the lines investigated have been found to contain such genes. On the other hand, very few seedlings, *i. e.* usually less than 100, have as a rule been studied from each plant, and a second generation originating from plants giving no recessives in their first isolated generation has usually not been tested previously. VALLE (1931) points out, however, the fact that among the lines he studied, some gave white seedlings in I_2 in spite of the fact that their mother plants had not given any such seedlings. Taking all these facts into consideration, it is likely that chlorophyll deficiencies usually are very common in cultivated *P. pratense*.

Albino seedlings as well as different kinds of yellowish chlorophyll deficiencies have appeared in the material tested in the present investigation. It is, however, only the white ones that have been classified, in order to make the study less intricate. The mode of inheritance

which this character exhibits, seems to be of a polymeric type. This fact explains that the plants are able to conceal such a high frequency of genes for a lethal character in a fully vital population. As *P. pratense* is a hexaploid, the hexasomic mode of inheritance would be the most likely. Nothing in the segregation ratios obtained contradict this assumption. Almost all segregation ratios observed in plants of the families studied fall into regions which would be expected with a hexasomic inheritance. The segregation ratios obtained show, however, that many of the investigated plants must contain more than one set of genes for albino seedlings, the majority of the studied plants giving ratios which have been interpreted as derived from a complex hexasomic segregation. Only a few plants yielded segregation ratios somewhat deviating from those expected by hexasomic inheritance, and those plants were all rather self-sterile, why disturbances in their segregation ratios are to be expected.

The occurrence of a tetrasomic inheritance in four of the six genomes is a possibility which has been thoroughly studied by MYERS (1944). With the limited material he had, most of the segregation ratios he found in the families could be explained by both the hexasomic and the tetrasomic mode of inheritance. One of the plants, however, can only be explained by some type of hexasomic segregation. Many of the plants in the present investigation have given segregation ratios which cannot be derived from any tetrasomic mode of inheritance. This is especially the case with the plants referred to the triplex group. The segregation ratios of the first generation of the plants Nos. 4, 14, and 15 show a statistically significant deviation from the tetra-disomic ratio 1 : 143 ($P < 0{,}015$). The same is the case with several triplex I_1 plants of those families. So have two I_1 plants of No. 4 given 5 : 1976 and 4 : 2148, one I_1 plant of No. 14 yielded 2 : 1234, and one I_1 plant of No. 15 gave 2 : 1032, all ratios which hardly can be derived from 1 : 143. As no marked tendency for multivalent formation is found in meiosis, no higher degree of chromatid assortment is likely to occur, and thus no segregation ratios with less recessives than 1 : 143 would be expected to appear. Plant No. 18 also yields a segregation ratio which hardly can be derived from any tetra-disomic mode of segregation. Even if the other duplex plants Nos. 8 and 13 statistically might be derived from the tetra-disomic ratio 1 : 28,0, this derivation is, however, not very likely since a strong excess of recessives must be postulated in both cases. From a biological point of view, a deficit of white recessives is rather to be expected. Moreover, the tetra-disomic

segregation ratios from which some families of the material here investigated can be derived always seem to belong to only a few certain genetical types, the segregation pattern of which always is very similar to a hexasomic one. No family has been derived from any tetra-disomic constitution, the segregation ratio of which is not similar to a hexasomic one.

From the disomic mode of inheritance, very few if none of the families studied could be derived. The few plants giving ratios of the first generation which statistically might be derived from a disomic one are plants Nos. 3, 9, and 16. All those plants are among the most self-sterile ones, which throughout have given a very poor material. That is the reason why it has not been possible to test more than a few plants of the second generation. Single of those I_1 plants have, however, yielded a sufficient number of seedlings to show a segregation ratio which deviates statistically from any calculated disomic one. Thus, the segregation ratio of the parental plants seems to be disturbed by interfering sterility, since in such I_1 plants, where this interference is less pronounced, the segregation ratio is of a polysomic type. MYERS (1944) also came to the same conclusion, *viz.* that no disomic inheritance could be traced in the plants of *P. pratense* he studied. Also the polymeric inheritance with four factors and disomic segregation, giving a ratio of 1 : 255, in the first generation, could not be statistically proved in the second.

As a result of the data obtained in the present investigation, it may be concluded that the most likely mode of segregation for the hexaploid *P. pratense* seems to be the hexasomic one. Since genes for albino seedlings are very common in the strain of *P. pratense* studied, it seems as if almost every plant holds such genes in more than one locus, that is to say, that the majority of the plants have genes for white seedlings in more than one of their seven chromosome »sextettes». This system will give the plants a very intricate mode of segregation. Moreover, it makes it possible for the plants to conceal a great number of lethal genes in a vital population. And the possibility of obtaining homozygous plants by inbreeding is made almost impossible by this type of segregation. Firstly, the generations needed for obtaning homozygosity are far more numerous by a polysomic than by a disomic inheritance; secondly, the large number of lethal and semi-lethal genes, which must be concealed in a population with polysomic inheritance, will disturb the vitality of the inbred progeny.

As a consequence of the polysomic mode of segregation in the

hexaploid *P. pratense*, it must be assumed that the six genomes of this species are more or less homologous and that the corresponding chromosomes from these genomes are able to pair at random in spite of the fact that only solitary multivalents are formed during meiosis.

Acknowledgement. — The author wants to express her gratitude to Professor G. BONNIER for helpful criticism as to the statistical calculations in the present investigation.

SUMMARY.

(1) The occurrence of white seedlings after isolation has been studied in 21 plants of hexaploid *Phleum pratense*. The investigation has been carried out in both the first and, the self-fertility of the plants permitting, in the second generation.

(2) The segregation ratios obtained from the families studied have been compared with theoretically expected ratios calculated for three different types of hexaploids. (a) Hexaploids with a disomic segregation and reduplicated genes in corresponding chromosomes giving polymeric inheritance. (b) Hexaploids with a tetra-disomic segregation, *i. e.* four of the six genomes with a tetrasomic segregation and the remaining two with reduplicated genes in corresponding chromosomes but with a disomic segregation. (c) Hexaploids with a hexasomic segregation. Only the recessive homozygotes were assumed to give white seedlings, all the different kinds of heterozygotes to give green phenotypes. The calculation was made for two different loci of two separate chromosome »sextettes».

(3) The segregation ratios obtained for the different families revealed the hexasomic mode of segregation as the most likely and in some families as the only one explaining the observed ratios. The disomic segregation did not explain the inheritance of any family, where it was possible to test both studied generations. Nor could four polymeric factors with independent disomic segregation explain the mode of inheritance in any family.

(4) Every plant from the studied »Omnia» strain of timothy, which has been tested in two consecutive generations (20 individuals), contained genes for white seedlings, and the majority of the plants seem to hold such genes in several loci.

(5) The very complicated system of segregation represented by the hexasomic type, which the investigated families seem to perform, makes

it possible for the plants to conceal a great number of lethal genes in vital populations, as has been demonstrated in the studied strain of »Omnia» timothy.

(6) A consequence of the polysomic mode of segregation, demonstrated in the plants of *Phleum pratense* studied, is that the corresponding chromosomes of the different genomes seem to be able to pair at random, and, thus, all six genomes of the species ought to be more or less homologous, in spite of the fact that multivalents only rarely are formed.

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EFFECTS OF X-RAYS ON THE PRECIPITABILITY OF DIPHTHERIA TOXIN

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THE genetic and physiologic effects of ionizing radiations are the subject of a series of investigations (*cf.* EHRENBERG *et al.*, 1952; NYBOM *et al.*, 1952; and literature cited in these papers). An understanding of the mode of action of the radiations requires knowledge of their effects on substances of biological importance, especially the proteins. Since antigenicity of proteins has been claimed to be related to specific properties of genes and chromosomes (EMERSON, 1944; STURTEVANT, 1944), we felt that some importance would attach to an investigation of the effects of irradiation on antigenic properties of proteins.

Diphtheria toxin preparations (containing 40—80 I.U. per ml.) and specific horse sera (800—1500 I.U. per ml), obtained from the National Bacteriologic Laboratory, were irradiated with X-ray doses within the range of 200—30000 r (X-ray tube run at 175 kV, no filtration). The activities of the treated toxins and sera were determined by the method of optimum precipitation (KABAT and MAYER, 1948, p. 59), consisting in determination of the antigen—antibody ratio at which the precipitation is most rapid. The constant antigen optimal point was used; in the case of diphtheria toxin it has been demonstrated by RAMON (1922) to be the neutral point.

The following can be said about the effects of irradiation on the proteins in question. The titer of the toxin solutions, *i. e.*, the serum concentration at which flocculation first appears, is decreased to about two thirds of the orginal value at X-ray doses of (500—)1000 (—3000) r (Fig. 1). At higher doses — 1000—30000 r — the precipitin titer is restored progressively so that at 25000—30000 r it may be identical with that of the untreated toxin; in these cases precipitation occurs a few minutes later than it does in the untreated standard, at precipitation times of 15—20 mins. The X-ray effect on toxin is remarkable in that the whole of the *precipitation time-serum concentration* curves are shifted, *i. e.*, with an excess of toxin the irradiated toxins pre-

cipitate more rapidly than the controls whereas with an excess of serum the untreated toxins are the better. The magnitude of the effects on toxins is, in some degree, dependent on unknown factors in the toxin preparation. It could be demonstrated to be independent of the toxin

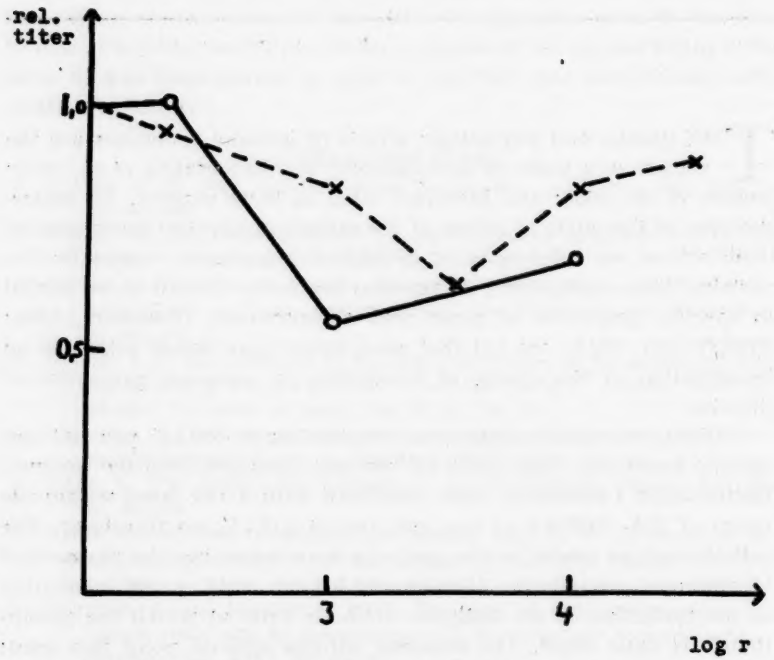

Fig. 1. Precipitin titer of diphtheria toxin in relative units as a function of log irradiation dose in r units. Two experiments.

concentration, because vacuum-dried toxin reacted in the same way as dissolved toxin.

In irradiation of antibodies (*i. e.*, the total specific horse serum), we were unable to find a similar effect. Antibodies irradiated with 200—30000 r were identical with the untreated serum.

β-rays from phosphorus-32 were found to exert an effect similar to that of X-rays on the toxin. Their effect on serum has not yet been investigated.

It is impossible to discuss, on the basis of the present data, the nature of the effects. Further experiments on less complicated, purified

antigens, involving complement fixation power and molecular weight determination, will make clear how much the specific reaction or the (secondary and slow) precipitation (*cf.* EHRENBERG *et al.*, 1946) is involved.

An effect of irradiation that becomes reverted with increasing doses is no unique phenomenon. The radiation effects on antigens reported here are superficially reminiscent of those on different enzymes in barley (NILOVA, 1936; FORSSBERG, 1943). Following irradiation *in vivo* several enzymes exhibit minimum activity at about 12000 r. The bouillon components of the toxin solutions are biologic in origin and may have an influence similar to that of the surrounding tissue in the case of barley enzymes. Several investigations by other workers have shown that the immunizing properties of antigens from different sources are unaffected even at very high radiational doses, *e. g.*, inhibiting the reproducibility of bacteria and viruses (see BONÉT-MAURY *et al.*, 1943; LEVADITI *et al.*, 1943). The immunizing power of tetanus toxin and staphylococcal hemolysin (EPHRATI, 1943) is left unaltered at doses of the magnitude used in the present investigation. The present effect, therefore, probably is not an alteration of specificity but may be related to changes in size and/or charge of the toxin molecules. (Precipitability decreases with decreasing molecular weight of the antigen; a low-molecular weight hapten is quite devoid of precipitability.) The irradiation of protein and other sols is known to induce changes with maxima and minima in particle size and charge (DESSAUER, RAJEWSKY; *cf.* FORSSBERG, *l. c.*).

The outstanding insensitivity of the immunologically specific properties of the proteins, diphtheria toxin and its horse antibody, indicates that such properties are not connected with readily oxidable groups, such as sulfhydryl. *From a genetic point of view, this insensitivity of specific protein properties must be considered in the discussion of the mutagenic effect of ionizing radiations.*

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IRRADIATION EFFECTS, SEED SOAKING AND OXYGEN PRESSURE IN BARLEY

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IN a series of papers the present authors have tried to unveil some of the happenings in X-ray and neutron irradiated seeds of cereal species (*cf.* the literature list for an information on this point). By various pre- and post-treatments the reaction of, say, a barley seed to irradiation can be fundamentally changed, resulting in an increase or a decrease of germination, seedling growth, chromosome disturbances and mutation rates. We hold the opinion that X-rays and neutrons exert their effects in different manners, neutrons chiefly by a direct action on the chromosome material, X-rays by means of combined direct and indirect effects. The end result of the irradiation will differ widely, depending on the actual metabolic state of the irradiated seed, temperature and oxygen pressure during irradiation, as well as the ionization density of the agent used. We may continually follow a set of properties and responses, beginning with enzyme content of the irradiated seed, immediate effects on the chromosomes, germination, seedling growth, plant fertility, and ending finally with mutation types and rates in the second or third generation.

Here, we shall discuss some experiments relating to the title of the paper, and concentrate on a few of the effects studied.

THE INFLUENCE OF SEED SOAKING CONTRA SEED DORMANCY.

Since STADLER's discovery in 1928 that in barley an onset of germination previous to the irradiation greatly increases mutability, this fact has been repeatedly discussed in the mutation literature. Often the results have been somewhat ambiguous, however. See, for instance, GUSTAFSSON and NYBOM, 1949, Table 1; MAC KEY, 1951, Table 3; EHRENBERG *et al.*, 1952, Table 4. In order to settle this discrepancy the present authors examined in 1951 and 1952 on a larger material the X_2 mutation rates of chlorophyll lethals, starting with the low dosage

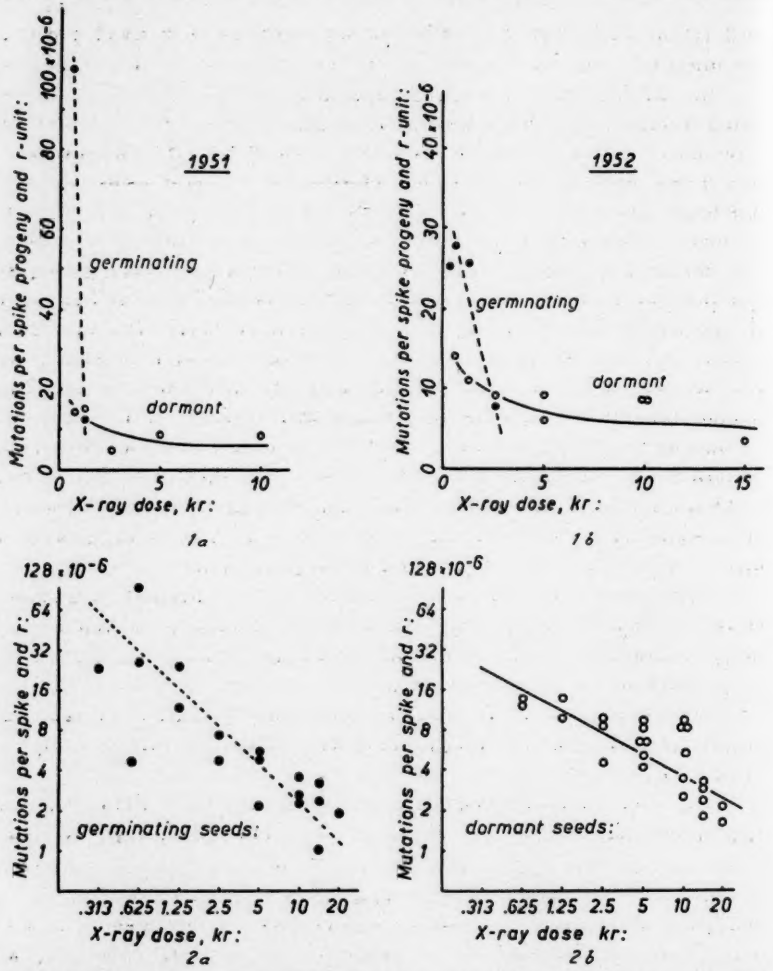

Fig. 1. Mutation rates of presoaked (germinating) and dormant seed series. Experiments of 1951 (*a*) and 1952 (*b*). Calculated per spike progeny and r-unit. — Fig. 2. Mutation rates of presoaked (germinating) and dormant seed series 1948—1952. *a* Soaked seed series. *b* Dormant seed series.

of 313 r and ending with fully lethalizing doses. Fig. 1 immediately evidences that with low dosages the presoaked seeds reach higher levels of mutability than the dormant ones. As a matter of fact the mutation rate was in 1951 with a dose of 625 r seven times as high in soaked as

in dormant seeds. The difference was less pronounced in 1952, but still the mutation rate was two to three times higher in presoaked seed series.

In addition, there is a conspicuous decrease in mutation rate with rising dosage, calculated per r-unit and spike progeny. This is especially pronounced in the presoaked seed series. Sooner or later the mutation rate drops down to the level characteristic of dormant seeds. In 1951 this happened with 1250 r, in 1952 with 2500 r. This peculiar behaviour is further visualized in Fig. 2. Here the mutation rates of presoaked and dormant seed series, from 1948 and onwards, have been gathered. The dosages used cover a range from 313 to 20000 r. In these five years 18 presoaked and 25 dormant seed series have been examined with regard to second generation effects. The results, of course, vary from year to year, with the mode of presoaking, the methods of irradiation, temperature, field condition, *etc.* Nonetheless, there is a dinstinct difference with regard to the slopes of the regression lines in the two types of seed used. The maximal mutation rate of the presoaked seed series reaches the level of $100 \cdot 10^{-6}$, *i. e.* six times as high as the maximal rate of dormant seeds. With very high dosages the presoaked seed series give low mutation rates, often lower than in dormant seeds.

Evidently there occurs a pronounced »intrasomatic selection» (KAPLAN, 1951; NYBOM *et al.*, 1953). This »selection» becomes even more conspicuous if the irradiation of presoaked seeds takes place in an atmosphere of oxygen (Fig. 4).

The frequencies of chromosome disturbances, analysed in the first mitosis after irradiation, contrast strikingly to the decrease in mutation rates (Table 1).

The high mutation rates with low dosages (313—1250 r) in the case of presoaked seed correspond to profound chromosome disturbances. In fact, 625 r give with preosaked seeds the same disturbance rate as 10000 to 15000 r with dormant seeds. The maximal amount of disturbed cells appears in presoaked seeds with 2500—5000 r, in dormant seeds not until doses of 15000 r or higher. With 2500 r the X_2 mutation rates lie at a similar level in both types of seed, below $10 \cdot 10^{-6}$; but there are 84 % of disturbed cells in the presoaked seeds as compared with 5 % in the dormant ones.

Quite a similar result is obtained in the analysis of plant fertility data (Table 2). With dormant seeds and 2500 r the average plant fertility is $92 \pm 0{,}7$ %, *i. e.* slightly subnormal. In presoaked seed it falls to $58 \pm 2{,}6$ %. The P-value for this difference is less than 0,001. However, the mutation rates of the X_2 generation are approximately identical.

TABLE 1. *Chromosome disturbances in the first mitotic division of presoaked or dormant seed series.*

(Experiments of 1952.)

	Dormant seed series			Presoaked seed series		
	% disturbed cells	% cells with bridges	No. of cells	% disturbed cells	% cells with bridges	No. of cells
Control	0,0	0,0	80	0,5	0,0	212
313 r	4,5	0,0	89	2,1	0,0	96
625 r	4,9	0,4	265	43,8	17,1	105
1250 r	2,8	0,0	145	16,5	6,2	339
2500 r	4,8	0,0	340	84,2	46,9	113
5000 r	6,8	0,9	220	96,1	50,0	52
10000 r	14,6	3,9	233	63,1	51,6	32
15000 r	60,3	24,4	31	—	—	—
20000 r	57,1	23,1	238	—	—	—

OXYGEN PRESSURE AND X-RAY EFFECTS.

In the publication of NYBOM *et al.*, 1952, the methods employed have been fully discussed.

We first consider the irradiation effects on presoaked seed in nitrogen atmosphere.

TABLE 2. *Average plant and spike fertilities of the X_1 generation resulting from presoaked and dormant seeds. Presoaking under different oxygen pressure during irradiation.*

(Experiments of 1952.)

	Presoaked seeds			Dormant seeds
Plants	Air	Nitrogen	Oxygen	
Control	97,8 %	97,7 %°	98,2 %°	97,2 %°
313 r	95,3±0,5	95,3±0,5°	90,4±0,7***	97,0***
625 r	81,0±1,3	91,9±0,6***	72,3±1,4***	97,6***
1250 r	76,7±1,3	79,4±1,3°	59,8±1,9***	95,0***
2500 r	57,6±2,6	55,8±1,7°	—	91,9***
Spikes				
Control	98,7 %	98,7 %°	98,7 %°	97,6 %°
313 r	96,3±0,4	95,7±0,5°	89,8±0,7***	98,2***
625 r	81,1±1,1	89,8±0,6***	71,1±1,2***	98,0***
1250 r	76,5±1,0	77,9±1,1°	61,5±1,5***	96,4***
2500 r	57,3±1,9	56,5±1,4°	—	92,9***

The X_1 damage is less pronounced with nitrogen than with air (Figs. 3 and 4, and Table 2). There is a better germination in the field, a greater number of harvested plants and a higher average plant and

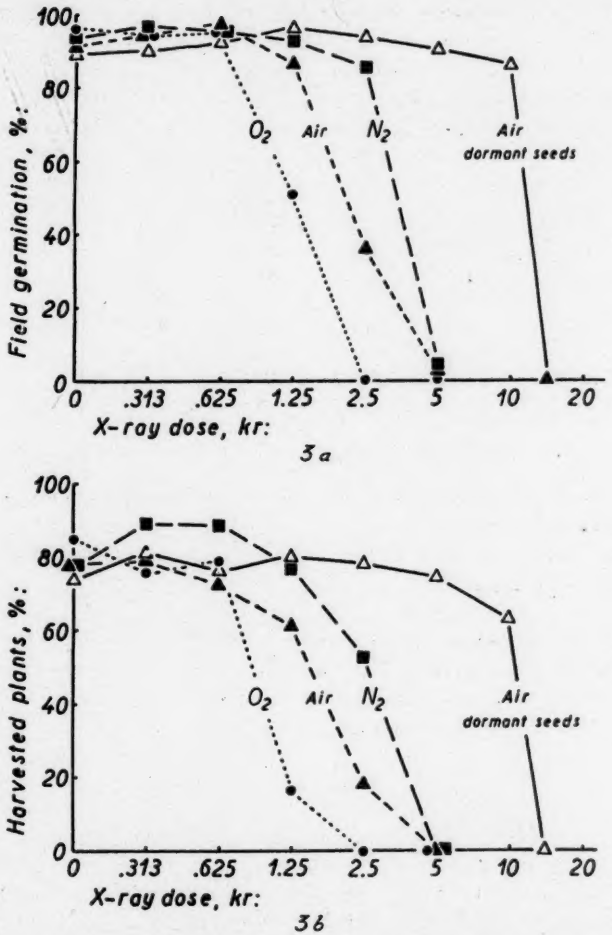

Fig. 3. Field germination after three weeks (*a*) and percentage of harvested plants (*b*) in different types of atmosphere during irradiation, compared with the behaviour of dormant seed series. Experiments of 1952.

spike fertility. The nitrogen atmosphere also exerts a distinctly beneficial effect with regard to chromosome disturbances at the first mitosis after irradiation. These results agree with the mutation rate of the second generation (Fig. 4). Nitrogen-treated seeds produce with 313, 625 and 1250 r fewer mutations per spike or plant progeny than seeds irradiated

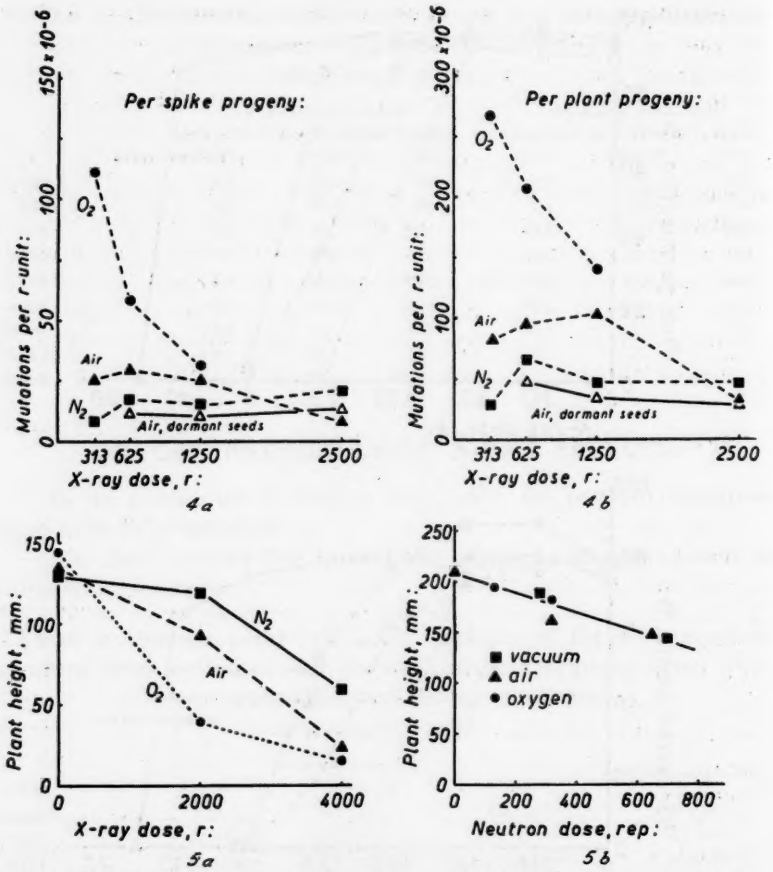

Fig. 4. X_2 mutation rates in different types of atmosphere prevailing during irradiation, compared to dormant seed series. Calculated per r-unit and spike progeny (*a*), per r-unit and plant progeny (*b*). Experiments of 1952. — Fig. 5. The influence of type of atmosphere during irradiation on seedling growth three weeks after germination. With X-rays (*a*) and neutrons (*b*). Parallel greenhouse experiments of 1952.

in the common manner (air atmosphere). With 2500 r, however, the mutation rate is slightly higher than in air.

On the other hand, the irradiation in an oxygen atmosphere greatly increases X-ray damage. This is so for most properties studied. There is no need to enter into all details. The figures immediately visualize

the conditions. They further illustrate the pronounced elimination in the case of an oxygen treatment. The mutation rate, calculated per spike progeny and r-unit, decreases from $113 \cdot 19^{-6}$ with 313 r to *circa* $30 \cdot 10^{-6}$ with 1250 r, where it almost drops down to the level of air-treated seeds. No X_1 plants survived the higher dosages.

In the papers of EHRENBERG *et al.*, 1952, and NYBOM *et al.*, 1952, it was shown that a nitrogen or hydrogen atmosphere, at the time of irradiation, gave a better seedling growth under greenhouse conditions and in the case of presoaked seeds than an air of oxygen atmosphere. The seedling growth is an appropriate measure of radiation damage. With the analysis of this publication we have found that in all examined X_1 and X_2 properties oxygen accentuates the injuries of X-irradiation compared with air and that nitrogen counteracts them.

NEUTRON AND X-RAY EFFECTS UNDER DIFFERENT OXYGEN PRESSURE.

In Fig. 5 it is shown that under greenhouse conditions an irradiation with fast neutrons influences the plant growth in a manner entirely different from what occurs with X-rays (*cf.* NYBOM *et al.*, 1952, p. 359). All types of atmosphere give values, lying on a straight line. There is no difference between vacuum- and oxygen-treated seeds on one hand and the air-treated seeds on the other. Contrasting is the behaviour after X-irradiation, where oxygen accentuates and nitrogen mitigates the irradiation effects. The conclusion holds true, therefore, also in this new experiment, that neutrons and X-rays act in different manners.

The neutron data on immediate chromosome disturbances, first generation fertility and mutation rates are, owing to poor field conditions in 1952, not sufficiently conclusive. However, they do not disagree with the greenhouse experiments. This is especially true with regard to the first generation fertilities.

DISCUSSION AND CONCLUSIONS.

We may postulate two modes of action for ionizing radiations: first a direct or localized one, where the ionizations occur in or in immediate vicinity of the structures of vital importance, and secondly a non-localized and diffuse ionization primarily in water molecules, giving rise to active principles, the radicals H, OH and HO_2 and prob-

ably also H_2O_2, which indirectly bring the radiation energy to the site of action.

For the sake of briefness the two modes of action are ususally referred to as the direct and the indirect one, though these terms are not strictly adequate.

Densely ionizing radiations, like α-particles and neutrons, which produce a large number of ionizations close to one another, act in a direct way and are highly efficient in damaging and changing certain larger cell structures such as the nucleus and the genetical material (as well as plastids and other cytoplasmic particles).

Sparsely ionizing radiations like X-rays, on the other hand, act at least partially in an indirect way *via* intermediate steps. Besides effects on the genetical material such radiations also have more by-effects on the extra-nuclear cell material resulting in a general physiological damage.

Now, the action of densely ionizing radiations is different from the others already from a chemical point of view. No oxygen need be present for the formation of hydrogen peroxide if water is irradiated with α-rays (*cf. e. g.*, BONÉT-MAURY and LEFORT, 1948, and BONÉT-MAURY, 1952) or neutrons (*cf.* ALLEN, 1948). When OH radicals are formed sufficiently close to one another they will react, independently of the oxygen concentration, according to the formula $2OH \rightarrow H_2O_2$. Also the high, localized concentration of ionizations will produce an excess of reactive radicals sufficient to exclude the influence of competing molecules, for instance introduced SH-groups.

Following X-irradiation these primary radicals, H and OH, will be more widely spaced permitting dissolved oxygen to intervene, primarily by favouring the reaction $H + O_2 \rightarrow HO_2$. The diffuse distribution of ionizations also permits the action of chemical protectors (*e. g.*, SH-compounds) and the radiation effect will to a large extent depend on the general radiosensitivity of the cell (varying, for instance, with the physiological activity).

As a matter of fact, the hydroperoxyl radical, HO_2, is the intermediate product, the formation of which is inhibited in the protective action of sulfhydryl reagents (ALEXANDER and FOX, 1952).

Regarding X-rays, it is interesting to note that even the induction of phenomena like mutations and chromosome changes, usually regarded as »hit events» out from the target theory, is influenced by chemical and physiological agents in a manner suggesting an indirect action of the radiation.

Our results on this point agree with the earlier reported effects of the oxygen pressure on chromosome aberrations (*e. g.*, GILES and RILEY, 1950), mutations in plants (*e. g.*, HAYDEN and SMITH, 1949), in *Drosophila* (*e. g.*, BAKER and EDINGTON, 1952) and in microorganisms (ANDERSON, 1950). The latter author found one mutation to be dependent on the oxygen pressure, whereas another one was not. This finding is significant also to the plant-breeder, since it indicates a method of influencing the mutation rates of specific loci.

Usually there has been reported a saturation of the oxygen effects with an external ogygen concentration of around 20 % O_2, *i. e.* corresponding to the normal oxygen content in air. In our experiments, however, we obtained a marked increase in the radiation effects with the change from air to pure oxygen, which is perhaps due to the high oxygen consumption of the germinating seeds. In one special experiment we found no effect of stagnant oxygen, a continuous gas flow being necessary.

A negligible influence of the oxygen pressure in the case of the densely ionizing neutrons indicates a direct or localized action of the radiation. The findings, reported in this paper, fall entirely in line with previous results, suggesting a differential mechanism of neutron and X-ray action (MAC KEY, 1951; EHRENBERG and NYBOM, 1952; NYBOM *et al.*, 1952; and CALDECOTT *et al.*, 1952). THODAY and READ found in 1949 a much smaller influence of oxygen on the amount of chromosome aberrations produced by α-rays than by X-rays. Similarly, GILES *et al.*, 1952, stated that chromosome aberrations were 2—2,5 times as numerous in an oxygen than in a nitrogen atmosphere, when X-rays were applied, but that this proportion sank to 1,3—1,4 with fast neutrons and became unity with α-rays. In accordance with these results, FORSSBERG and NYBOM (1953) found sulfhydryl reagents to be less protective in the case of α- than of X-rays.

The radiation-induced effects obtained in presoaked seed can be considered from a similar point of view. The physiological state of the cell exerts a considerable influence (NYBOM *et al.*, 1952). The result is significant that the difference between dry and presoaked seeds in all properties examined is much smaller if neutrons than if X-rays are applied (EHRENBERG *et al.*, 1952). The effect of a presoaking on the frequencies of chromosome aberrations and mutations implies that the chemical milieu or other conditions of the chromosomes are somehow changed. The fact that this change more strikingly affects the results of X-irradiation than of neutrons, shows that especially with X-rays

the chemical milieu contributes to the radiation-induced effects. A precise statement concerning the true nature of this change cannot be given at present.

The conspicuous drop of mutation rates in the oxygen series with a rise in dosage makes an interpretation of the elimination processes possible. In our previous paper (1953, p. 450) we pointed out that an »intrasomatic selection» generally occurs in dormant seed series and is even more pronounced in soaked series. However, this elimination was less marked when the irradiation was carried out at the temperature of liquid air. This is in agreement with the postulated more direct action of X-rays at low temperatures. Similarly, MAC KEY (1951), EHRENBERG *et al.* (1952) and NYBOM *et al.* (1952) showed that the elimination processes were less profound in neutron than in X-ray series (*cf.* also CALDECOTT *et al.*, 1952). In this paper we have further indicated that an irradiation in a nitrogen atmosphere results in no detectable elimination but that an oxygen atmosphere drastically accentuates it (Fig. 4). The processes of elimination apparently decrease in the following order:

Presoaked X-rayed seeds, O_2 atmosphere.
Presoaked X-rayed seeds, air atmosphere.
Dormant X-rayed seeds, air, $+20°$ C.
Dormant X-rayed seeds, H_2S atmosphere, $+20°$ C.
Dormant X-rayed seeds, air, $-190°$ C.
Presoaked X-rayed seeds, nitrogen atmosphere.
Dormant neutron treated seeds.

The conclusion is close at hand that the indirect radiation effects decrease and the direct ones increase in relative importance according to the order mentioned. The following interpretation of the intrasomatic selection then seems to us to be plausible:

(1) Chromosome aberrations and re-arrangements are not responsible for the chief part of the elimination, since the elimination is negligible after neutron irradiation, also when this produces very high rates of chromosome disturbances and leads to a profound first generation sterility.

(2) Evidently the processes of elimination are easily influenced by various physiological factors (presoaking, oxygen pressure, temperature). Furthermore, the influence of a presoaking is less marked in the case of neutron than of X-ray irradiation. These facts suggest that the elimination is due to indirect effects of the radiation connected with damage of the plasma constituents.

(3) A mechanism, like the one suggested, implies that a great number of identical changes take place in the cells, or groups of cells, of the irradiated embryo. If we bear in mind that it is possible to influence both the radiosensitivity and the degree of elimination by physiological means, we may state that the individual cells, groups of cells, spike primordia, *etc.* will vary with regard to their physiological status. This in turn implies that they show a variable degree of radiosensitivity.

(4) When low doses are applied, chromosome aberrations and mutations increase with the dose and with a yield per dose unit that is determined by several factors, *e. g.*, metabolic state, oxygen pressure, temperature. With higher doses, however, there will be a reduction in the frequency of observable genetical changes due to a cumulative physiological killing of the most radiosensitive cells. The result will be a removal of chromosome aberrations and especially of mutations, an »intrasomatic selection», that simulates a process of recovery from genetical damage.

This picture of the elimination processes well agrees with the different mode of plant germination and growth after neutron- and X-irradiation, especially in the field (NYBOM *et al.*, 1952, p. 360). After neutron irradiation plant elimination does not occur until the stage when mitosis becomes of supreme importance for the continued growth. With high neutron dosages young plants may still be abundant, but they do not pass the seedling stage. After X-irradiation, on the contrary, plant elimination takes place at a very early stage, already at the time of germination, but plants that sprout overcome their radiation damage more readily than in the case of neutrons and survive.

Summarizing the actual facts we state: *that* a presoaking previous to a low dosage of X-irradiation distinctly increases the amount of chromosome aberrations, plant sterilities and mutation rates, *that* with high dosages elimination processes cause a considerable drop in mutation rates, calculated per r-unit, *that* a change in oxygen pressure at the time of irradiation has a profound effect in all properties examined, and *that* neutrons, finally, act in a manner quite different from X-irradiation. We may also state *that*, in order to obtain conclusive data on mutation rates and mutation types, the irradiation should be performed with low dosages, where the »intrasomatic selection» is not at work. This becomes especially important when we want to influence and direct the mutation process by experimental means.

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THE SPERMIOGENESIS OF THE BULL FROM A KARYOLOGICAL POINT OF VIEW

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THE present paper concerns karyological investigations carried out in order to elucidate chromosome conditions during spermiogenesis in the bull. The studies were made utilizing squashes and sections produced by improved methods. KRALLINGER (1931) and MAKINO (1944) obtained good results in earlier investigations of cattle chromosomes using the paraffin-section method. A review of the earlier literature on spermiogenesis in the bull was made by MAKINO (1944).

Both the authors just named were in agreement that the bull has a complement of 60 chromosomes, consisting of 58 autosomes+X+Y. KRALLINGER suggested that the X-chromosome is metacentric; he could not define the Y-chromosome.

MATERIAL AND METHODS.

The testicular material used for these investigations came from 31 bulls of the Swedish Red and White Breed (SRB). Four were 2—7 months old, 24 ranged from 8 months to 2 years in age, and 3 were about 6 years old.

It was possible to examine the bulls under 2 years of age since the rearing of young animals for slaughter became common in 1952. Bull calves were bred and never used for service, but were castrated in the spring of 1952 before pasturing. It involved only animals which to our knowledge had never been ill, and no idea of the fertility of these bulls could be obtained.

The material was collected either by castration, during biopsy, or at slaughter. Castration occurred within 5 minutes of the application of local anaesthesia with 2 % pure xylocaine. Biopsy was done without anaesthesia. The slaughter-house material was taken immediately after stunning and before bleeding-out. Fixation was made within five min-

utes after the stunning shot. The material was collected either in the morning or after the midday-rest of the animals, which was most advantageous in view of the fact that the frequency of cell division was highest then.

For fixation a mixture of 1 part glacial acetic acid and 3 parts absolute alcohol was used; it was prepared immediately before fixation. Pieces of the size of rice-grains were taken from different parts of the testicle's spermiogenetic regions for fixation. In those cases in which chromosome preparations were not made at once, the material was stored up to 7 months in a refrigerator with excellent results.

Both squash and section preparations were prepared for investigation. Paraffin sections were cut 7 μ thick and stained with GOMORI's haematoxylin, according to the schedule of MELANDER and WINGSTRAND (1953). Squash preparations were stained by the same method and pressed between cover- and object-glasses treated with silicon (Dri-Film 9987, from General Electric Co., N. Y.). Pressed preparations were frozen with carbon dioxide snow, after which it was possible to remove the cover-glass with a razor blade without damaging the underlying tissue. The preparations were mounted in Canada balsam after treatment with 96 % and absolute alcohol.

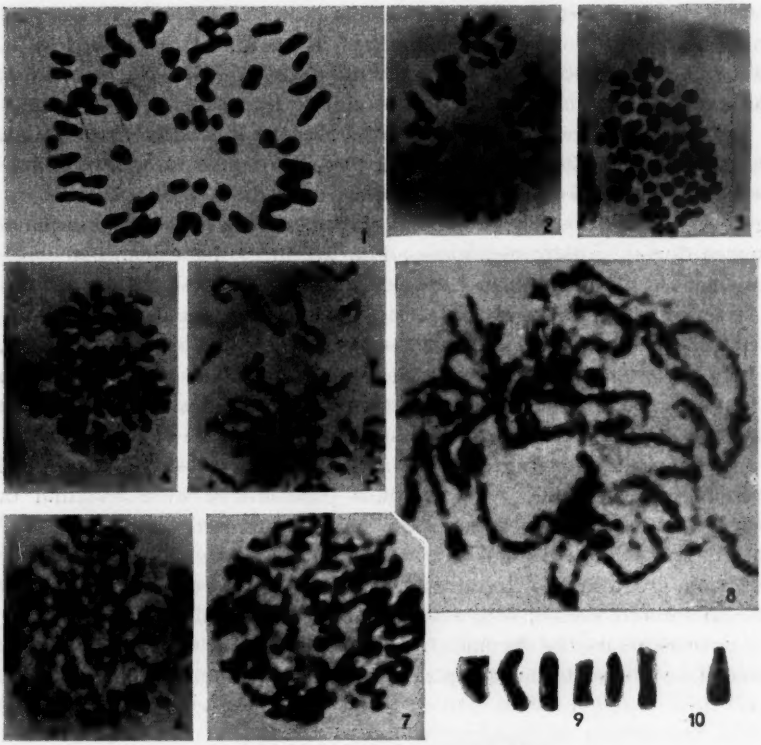
In both squash and section preparations damaged nuclei are always present. In view of this we have excluded such nuclei from chromosome studies. All chromosome counts were made by both authors personally.

RESULTS.

THE SPERMIOGONIA CELLS.

Interphase nuclei in spermiogonia cells are found immediately adjacent to the basal membrane of the tubulus. They are relatively large and generally supplied with a single nucleolus. In addition, two strongly stained grains, presumably nucleolus organizors from one pair of chromosomes, are found in these interphase nuclei.

When first discernible, chromatids are oriented in a bouquet with its basal end lying near the nuclear membrane, and the other end at random in the nucleus. No definite end-chromomeres are visible at the base of the bouquet but are observed on the free ends of the chromatids. If one makes the most probable assumption, namely, that the chromatids in the mitotic interphase are arranged in the same manner as they were during the previous telophase, the centromere



Figs. 1—2. Spermiogonial mitotic metaphase chromosomes, 60 in each plate. — Figs. 3—5. Stages of promeiotic contraction. The chromatid spirals uncoiling from Fig. 3 to 5. — Figs. 6—7. Zygotene (6) and late pachytene (7) bouquets. The chromocentres at the bottom of the photos. — Fig. 8. Late pachytene chromosomes in a bouquet flattened during preparation. Note the dark chromocentre at the lower part of the photo. — Figs. 9—10. Different types of bivalents; diakinesis. Fig. 10 is the *X*—*Y* bivalent with the *Y* at the top. — Magnif. 2000×.

must be situated in the weakly stained chromatid ends at the base of the bouquet.

During the spermiogonial metaphase (Figs. 1 and 2) most chromosomes are arranged in a ring, with the smallest lying innermost. The centromere ends point towards the centre of the ring. Of the 32 bulls examined, 31 definitely had a chromosome count of $2n=60$ in spermiogonial mitosis, so this number may be regarded as characteristic of the breed. Only animals with $2n=60$ were considered in this work.

A total of 101 60-chromosome plates were counted. These comprised 7 plates from each of 2 bulls, 5 from 3 bulls, 4 from 8 bulls, 3 from 9 bulls, 2 from 4 bulls, and 1 each from the remaining 5 bulls.

In a single cell in each of two bulls the deviating chromosome numbers 20 and 28 were found. In these two cases the chromosomes were abnormally contracted and not oriented on a plate.

It was not possible to identify with certainty any of the chromosomes during mitotic metaphase. The length of the chromosomes diminishes continually from the longest to the shortest, all the centromeres are situated terminally, and secondary constrictions are absent. At mitotic metaphase the chromosomes were somewhat variable in length. The longest chromosomes were 2,1—2,5 μ long, while the smallest chromosomes in the complement were about 0,7 μ long. The width of the chromatid spiral was about 0,6 μ.

From the appearance of the metaphase chromosomes it was not possible to determine whether their centromeres were terminal or slightly sub-terminal. At anaphase all chromosomes migrate simultaneously towards the poles, with the pointed ends first. The spindle threads are attached terminally, in so far as we could determine (from sections stained with acid fuchsin).

At telophase the chromosomes contract further with no observable uncoiling of the chromatid spiral.

THE PRIMARY SPERMIOCYTES.

It is apparent from studies of sections that primary spermiocytes develop only from daughter cells of the spermiogonia which are for some reason displaced inwards towards the tubuli lumen. Those remaining undergo mitosis. This can be demonstrated only because primary spermiocytes in bulls are easily recognized. (See below.)

Just inside the spermiogonia cell layer there are often many primary spermiocytes lying together. These have a characteristic appearance in bulls (Fig. 3). The nucleus contains 60 strongly contracted, nearly globular, highly stained chromosomes spread throughout the entire nucleus. These must come directly from the telophase chromosomes of the spermiogonia cells, unless some uncoiling of the chromatids occurs meanwhile. This stage of contraction of the telophase chromosomes in bulls corresponds to the diffuse pro-meiotic interphase stage in the majority of mammals in which the chromosomes uncoil as early as

telophase. Judging by the high frequency of such nuclei, the duration of this highly contracted stage is very long.

During transition to meiotic prophase, chromatids assume diffuse outlines as the chromatid spirals uncoil (Figs. 4 and 5). One end of each chromatid remains deeply stained.

Following uncoiling and loss of staining ability, the chromosomes are orientated with weakly stained centromere ends in a bouquet base, inside a restricted area near the nuclear membrane. The distal, strongly stained end-chromomeres lie at random in the nucleus. This orientation resembles that of spermiogonial interphase.

During the early meiotic prophase many of the chromatids aggregate at their centromere ends in one or several chromocentres which are stained somewhat more weakly than, for example, the distal chromomeres of the metaphase chromosomes (Figs. 6—8).

Zygotene pairing generally appears to begin at the base of the bouquet.

At pachytene the bivalents are arranged in a well-defined bouquet. In many organisms the peripheral bivalent ends with obvious terminal chromomeres bend towards the base of the bouquet, but this does not occur here (Fig. 26).

At diplotene the paired chromosomes separate from each other at both ends of the bivalents, to the extent allowed by the chiasmata. In the diplotene bouquet, the *X*—*Y* bivalent can be distinguished, as its centromere ends are pointed towards the base of the bouquet (Fig. 12).

During the co-orientation of pro-metaphase the centromere ends of the partially contracted bivalents are stretched towards the poles, as far from each other as the chiasmata allow (Figs. 13—15). The bivalents are arranged in a ring during this stage.

At full metaphase the bivalents shorten more obviously and rapidly and are nearly globular at the end of the congression period. The diameter of the largest bivalents is then about 2 μ. At the end of metaphase the bivalents are arranged randomly in a plate (Figs. 17 and 18).

Each bivalent has one to three chiasmata which usually lie at random along the chromosomes, though chiasmata tend to be more frequent at the centromere ends. Terminalization of chiasmata does not appear to occur before metaphase. The appearance of the bivalents during the diplotene stage and the pro-metaphase is shown in Figs. 9—11.

Bivalents in which chiasmata occur in the immediate neighbour-

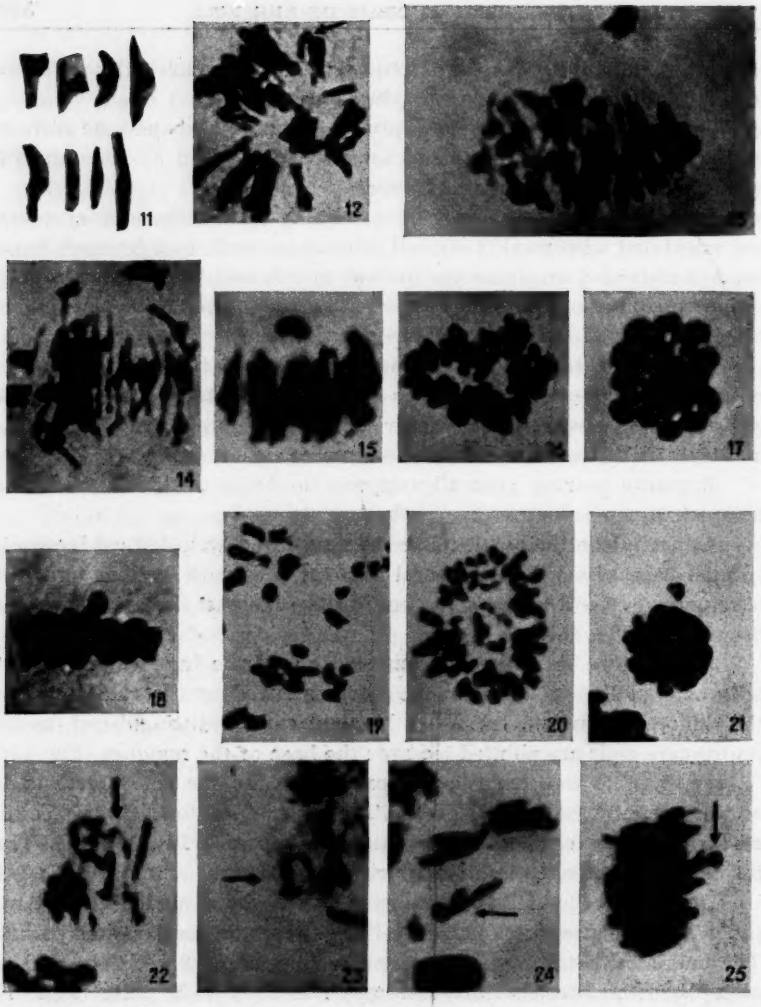


Fig. 11. Different types of bivalents, pro-metaphase. — Fig. 12. Somewhat flattened (by preparation) bouquet at diakinesis viewed from above. Note the *X*—*Y* bivalent at the arrow with the centromeres of both *X* and *Y* pointing towards the centre. — Figs. 13—16. Pro-metaphases. Note the bivalents at the top of Figs. 13—15, having difficulties in co-orientation because of a chiasma close to the terminal centromeres. — Figs. 17—18. Metaphase of the first meiotic division. — Fig. 19. Anaphase chromosomes in the first meiotic division. — Fig. 20. Second meiotic division, metaphase. — Fig. 21. Prophase of the second meiotic division with V-shaped chromosome configuration lying outside. — Figs. 22—23. The pachytene *X*—*Y* bivalent at the arrows. — Fig. 24. The *X*—*Y* bivalent at diplotene. — Fig. 25. An uncommon type of *X*—*Y* bivalent at metaphase. The *Y* pointing transversally to the axis of the spindle. — Magnif. 2000×.

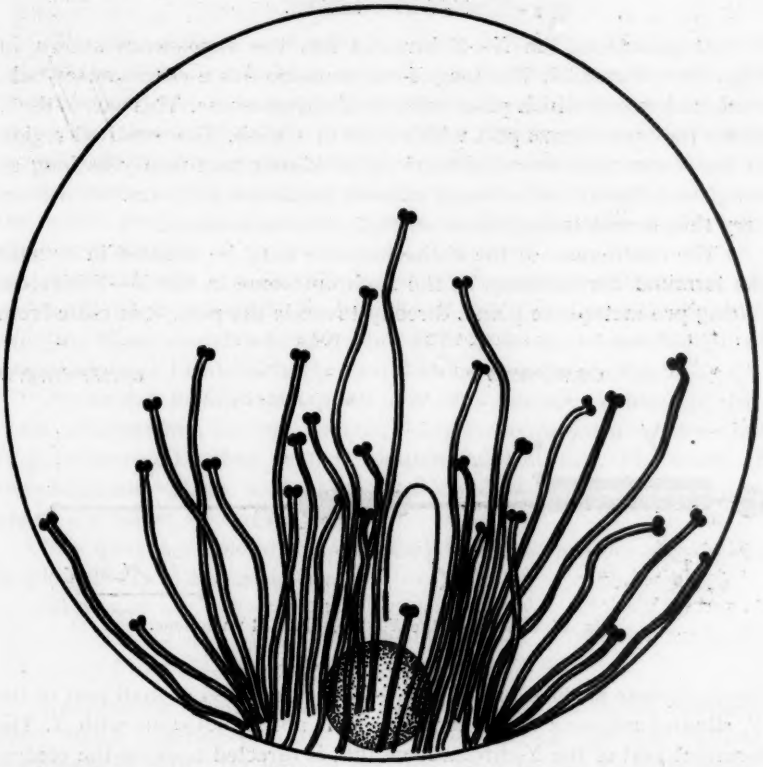


Fig. 26. Scheme of the orientation of bivalents at the prophase of the first meiotic division. The centromere ends point to the dividing centrosome with the opposite ends distributed at random. The *X*—*Y* bivalent in the middle. The chromocentre at the bottom.

hood of the centromeres have the appearance of a »V». Such bivalents have obvious difficulties in co-orientation. They are often found during pro-metaphase lying well outside the plate, as is sometimes the case at full metaphase (Figs. 13—15). It is possible that some of these bivalents do not divide during the first meiotic division, but are incorporated undivided into one of the daughter nuclei. This assumption appears to be supported by the fact that a »V»-shaped chromosome configuration with doubled chromatids in each arm is not infrequently seen lying outside the bouquet during the second meiotic prophase (Fig. 21).

X-Y BIVALENTS.

At pachytene the *X*—*Y* bivalent has the appearance shown in Figs. 22—24 and 27. The long *X*-chromosome has a chromomere-rich, thick end region which pairs with the *Y*-chromosome. This part of the *X* forms the homologous part which ends in a knob. The unpaired region of the *X*-chromosome consists of (a) a thinner part nearly as long as the paired region, (b) a deeply stained, thickened part, and (c) a long, very thin thread ending in an obvious end-chromomere.

The centromere of the *X*-chromosome must be situated in or near the terminal chromomere, as the *X*-chromosome in the *X*—*Y* bivalent during pro-metaphase points directly towards the pole. It is clear from

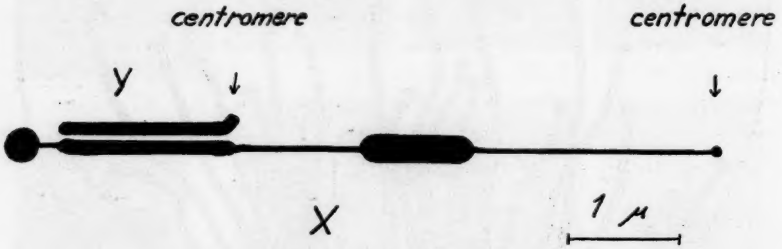


Fig. 27. Scheme of the *X*—*Y* bivalent at pachytene.

the pachytene morphology of the *X*—*Y* bivalent that a small part of the *Y*, situated adjacent to the centromere, is not homologous with *X*. The terminal part of the *Y*-chromosome that is directed towards the centromere end of the *X*-chromosome during early meiotic prophase must contain its centromere. If the *Y*-centromere had another position it would be difficult to explain the appearance during pro-metaphase of a thread-shaped *X*—*Y* bivalent, the *Y* end of which points directly towards the pole (Fig. 10).

A chiasma between the *X*- and *Y*-chromosomes is most often formed at their distal ends. At diplotene, when the autosomal bivalents lie arranged in a bouquet of straight rods, the *X*—*Y* bivalent is a thinner bent thread, both ends of which point towards the base of the bouquet (Fig. 12). Chiasmata in the *X*—*Y* bivalent were seldom observed in other than a distal position.

The *X*—*Y* bivalent divides simultaneosuly with the autosomal bivalents at anaphase, and not precociously as occurs, for example, in the mouse.

THE SECONDARY SPERMIOCYTES.

The second division of meiosis rapidly follows the first one. As at interphase of the first division, there is a stage with strongly contracted chromosomes which is very short, judging by its infrequent appearance. The chromatid spirals from the first division of meiosis rapidly uncoil. Later in the prophase of the second division there are bouquets of double chromatids arranged principally as in the preceding and also the mitotic prophases. However, their orientation cannot be seen equally clear at this stage.

As previously stated, a V-shaped chromosome configuration occasionally appears outside the bouquet of the second meiotic division; this we have associated with the difficulties in co-orientation of certain types of bivalents in the first division of meiosis (Fig. 21).

At second meiotic metaphase the chromosomes consist of two arms, attached by their centromeres. They are arranged in a very close ring, often smaller than that of spermiogonial mitosis. The width of the chromatid spirals is less than in the spermiogonial mitosis metaphase, *i. e.* about 0,5 μ (Fig. 20).

The daughter cells of the secondary spermiocytes, the spermatids, develop slowly to mature sperms.

DISCUSSION.

PREVIOUS LITERATURE.

KRALLINGER (1931) described »ein V-förmig gestaltetes Chromosom» which reached the pole precociously during the first meiotic metaphase in cattle. He implied that this must be the *X*-chromosome, but emphasized that he could not find any chromosome element at the opposite pole which could be *Y*. According to what has been said above, one may regard this as an autosomal bivalent with difficulties in co-orientation.

MAKINO (1944) described the *X*—*Y* bivalent at pro-metaphase. He mentioned the bending of the stretched bivalent's end, as we have also seen in individual cases (Fig. 25). With the interpretation of the *X*—*Y* bivalent we have given, one can obtain a picture like MAKINO's, if one assumes a chiasma in the homologous part which is not situated terminally.

THE CENTROMERE.

It has been concluded on the basis of the following evidence that the centromeres are situated in the chromatid ends which lack large

end-chromomeres. At mitotic prophase the large end-chromomeres lie evenly distributed in the nucleus. The assumed centromere ends are concentrated around a point inside the nuclear membrane, outside of which the centrosomes are situated. It is improbable that all the chromosomes should have reversed themselves during the beginning of the interphase. This orientation is more probably a relic of the previous division and, in addition, is probably caused by an attraction between centrosome and centromere.

The coiled chromatids in the stage of contraction preceding meiosis are spread throughout the nucleus and are later oriented in a bouquet. This can be interpreted by assuming that an attraction exists during the latter part of the meiotic prophase between centrosome and the presumed centromeral ends of the chromatids, although the nuclear membrane is still present. The attraction occurs noticeably towards the end of prophase in association with the division of the centrosome. The centromeres of the *X*- and *Y*-chromosomes also lie terminally and their centromeral ends both point towards the base of the bouquet at late meiotic prophase. There is no reason to suppose that the centromeres of the autosomes behave differently than those of the sex chromosomes during prophase orientation of the bivalents. It is probable that the centromeres of the autosomes also point towards the base of the bouquet. It is clear from the morphology of the *X*—*Y* bivalent at pachytene that the *X*-chromosome (and also the *Y*) has an obvious end-chromomere which must be located on the end opposite to the centromeric end. The autosomes and sex-chromosomes thus appear to have a similar morphology in these respects.

THE CO-ORIENTATION OF BIVALENTS.

Most chromosomal contraction in bulls occurs towards the end of pro-metaphase and at early metaphase. During late metaphase the chromosomes are strongly contracted. There is further contraction at telophase and in the succeeding contraction stages of interphase between the meiotic divisions. During co-orientation of the bivalents at pro-metaphase, the pulling force which acts on the centromeres is therefore able to separate the partially contracted chromosomal parts and stretch the bivalents appreciably towards the poles (Figs. 11 and 13—15). Thus a pro-metaphase stretch is observed, like that described by HUGHES-SCHRADER (1943, 1946, 1947) in *Mantids* and *Phasmids*. ÖSTERGREN (1951) discussed the general presence and significance of the phenomenon. As coiling and contraction usually occur at an earlier

stage of meiosis in other material, the pro-metaphase stretch is generally less pronounced than in bulls.

As mentioned above in the description of the first meiotic division, significant difficulties in co-orientation not infrequently occur for certain bivalents. If the centromeres are terminally located and kinetically active only in their terminal parts, the presence of a chiasma in their immediate neighbourhood might interfere with co-orientation of the bivalent, as the short free chromosomal parts between the chiasma and the centromeres may not be sufficiently flexible to allow the kinetically active centromere parts to be directed towards opposite poles. One might then have a parallel here with the lack of orientation of some mitotic chromosomes in *Ulophysema* which also have terminal centromeres (MELANDER, 1950). In both these organisms it is probable that the terminal centromere ends of the chromosomes are not sufficiently flexible under all conditions to permit perfect chromosomal orientation. The condition must be of a considerable importance in the bull where it can result in gametes with faulty chromosome complements. In *Ulophysema* the somatic chromosome number varies for the same reason. Probably these disadvantages in the chromosome morphology characteristic of cattle are counterbalanced by a very complete prophase orientation with resultant regular chromosomal pairing.

OTHER PECULIARITIES OF THE CHROMOSOMES.

Detailed morphological studies of bull chromosomes are nearly precluded by the small chromosomal size, their high number and uniform appearance.

An advantage in counting chromosomes is that all the chromosomes have terminally situated centromeres. Therefore, there is no doubt as to whether one is dealing with one chromosome with a median centromere, or two with terminal centromeres. One cannot recognize the different metaphase chromosomes, but it should be possible to identify the individual pachytene bivalents in preparations made according to the technique used here.

During the second meiotic division the chromosomes are very small and arranged in very small metaphase plates, so that a precise estimation of chromosome number is seldom possible. Otherwise this would have been of great interest in view of such a high incidence of difficulties in co-orientation. On the assumption that such bivalents migrate undivided to one pole during the first division of meiosis, one should find corresponding variations in the chromosome number in

the second division. Unfortunately, we were unable to confirm such variations.

Mammals in general have a more diffuse interphase before both the meiotic divisions. In contrast there occurs in cattle the contraction stage described above. According to our observations the same condition does not occur in sheep, and therefore the contraction stage is not characteristic of ruminants.

Owing to the uniform chromosomal morphology of the entire complement with constantly terminal centromeres, the bull material treated here appears to be well suited for continued studies on the mechanism of bouquet formation.

SUMMARY.

The authors give a review of chromosomal conditions during spermiogenesis in bulls of the Swedish Red and White Breed (SRB). The complement was confirmed as 58 autosomes $+X+Y$. All the chromosomes have terminally situated centromeres. True meiosis in spermiocytes is preceded by a contraction stage in the chromosomes. During certain parts of the mitotic interphase and the later part of the meiotic prophase a bouquet orientation occurs where the centromere ends of the chromosomes lie in the base of the bouquet. One or several chiasmata are formed anywhere along the autosomal bivalents, especially near the centromeres. The maximum extent of contraction in the bivalent chromosomes occurs only after the pro-metaphase, which causes an obvious pro-metaphase stretch. Bivalents with chiasmata near the centromeres have difficulty in co-orientation and, in some cases, are probably passed on undivided to either daughter nucleus, where they are occasionally found during the prophase outside the chromatid bouquet. The morphology of the X—Y bivalent is described.

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